

American Journal of Clinical Pathology

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American Journal of Clinical Pathology

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THE HORMONE TEST FOR PREGNANCY*

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The function of the ductless glands may be investigated by two principal methods. The first method may be illustrated by the effects of the loss of the gland by disease, experimental removal, or extirpation, in which case the resulting symptoms are assumed to be the result of a hypoactivity of the gland, or of its absence. The second method consists essentially in the introduction of the gland, or of its extracts or secretions into the body of an experimental animal in order to produce reactions, which may be comparable to those brought about by an exaggeration of the glandular activity.

The function of the secretion of the anterior lobe of the pituitary body has been investigated by both of these methods. However, it is only in recent years, that the technique of the experimental investigation has been sufficiently perfected to allow one to make any very definite conclusions in regard to the functions of the gland. Besides the difficulty of the technical procedures, the problem has also been complicated by the effect of the hormone of the anterior hypophysis on other glands, especially the gonads, whose modes of functioning were also incompletely understood. Furthermore it would now seem that there is not one hormone secreted by the anterior lobe of the hypophysis, but three or possibly four.

As far as we have been able to ascertain, Horsley¹⁴ was the first to publish regarding the experimental removal of the pituitary gland. He stated that he had removed the pituitary body from two dogs which were sacrificed at the end of five and six months respectively and that hypophysectomy led to no disturb-

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ing symptoms. A truly remarkable statement when we consider the number of investigators in the next twenty years who were unable to maintain life in hypophysectomized animals for a sufficiently long time to observe any symptoms of importance. In fact, the question then arose as to the necessity of the gland for the maintenance of life. Investigators from the time of Horsley until 1908 met with varying results.

Paulesco¹⁵ successfully evolved a technique, which was a decided advance over any which had been previously used, and with this technique he obtained a long series of hypophysectomies. Of these twenty-four proved to be total, the average duration of life of all cases was twenty-four hours. From a second grouping of seven partial hypophysectomies, he concluded that removal of the anterior lobe like total removal of the gland resulted in death of the animals, and that loss of the posterior lobe led to no appreciable disturbance. Thus the hypophysis was concluded to be an organ indispensable to life.

Crowe, Cushing and Homans⁴ conducted an extensive study of experimental hypophysectomy, from which they concluded that "total removal of the hypophysis leads inevitably to the death of the animal with a peculiar and characteristic train of symptoms which have been called cachexia hypophyseopriva." Death did not necessarily occur as rapidly as Paulesco claimed as the "puppies may remain in an apparently normal condition for at least three weeks before terminal phenomena appear."

Removal of the posterior lobe leads to none of the manifestations of cachexia hypophyseopriva. Removal of the anterior lobe alone produces these symptoms.

The most striking feature of removal of the anterior lobe is a state of adiposity accompanied by a secondary hypoplasia of the organs of generation in adults or by a persistency of sexual infantilism in case the primary hypophyseal deficiency antedates adolescence.

Recent experimental studies on rats, cats, and dogs have shown conclusively that the hypophysis is not essential to life in these animals, and that although their span of life after hypophysectomy may be shorter than that of the control, it is of sufficient

length to indicate that the removal of the pituitary has not resulted in death.

Aschner² published striking photographs of hypophysectomized puppies of various ages which had remained infantile in appearance and size until sacrificed. From which we may conclude that (1) the loss of the anterior lobe of the hypophysis is not incompatible with life; (2) removal of the anterior lobe of the hypophysis brings about a train of symptoms, the most important of which, so far as we are concerned at this time, is failure of infantile sex organs to develop, and atrophy of adult sex organs.

These symptoms bear a marked resemblance to Fröhlich's¹¹ syndrome, which is supposed to be due to hypopituitarism, and is characterized by infantile genital organs and sex characters, and an associated disturbance of fat metabolism in which there occurs a marked and rather characteristic deposition of fat. From these observations we may conclude that there is a definite interrelation between the anterior lobe of the hypophysis, and the sex organs.

Having obtained a more or less definite train of symptoms as the result of extirpation of the anterior lobe of the hypophysis, both in experimental animals and as the result of disease in man, the next logical step was that of substitution therapy. Oral administration was instituted by Wulzen^{21,22} and Goetsch¹² with contradictory results. Later workers, especially Evans and Long⁶ and Smith,¹⁸ have demonstrated that oral administration has no effect on the date of maturity, at least of rats.

Evans and Long⁶ prepared an extract of bovine hypophyseal substance which they injected into the peritoneum of adult white rats and succeeded in producing very definite changes in the reproductive organs.

The results of these injections were as follows:

1. Estrus was absent or inhibited at long intervals.
2. The uterus remained infantile, but the ovaries were twice the size of those of the control animals.
3. Histological examination showed the presence of very abundant lutein tissue in the ovaries and the formation of this tissue about the egg in unruptured normal follicles and in atretic follicles. Ripe normal Graafian follicles were

invariably absent. A powerful, specific stimulus to lutein cell transformation was thus present in this hormone.

These are the first definite results obtained from the injection of an extract.

While numbers of attempts had been made to observe the changes produced by whole living gland transplants, the results were unsatisfactory until Smith^{17,18} using daily transplants of anterior lobe succeeded in overcoming to a large degree the effects of hypophysectomy by restoring an almost normal growth rate. Thus, knowing the symptoms produced by ablation of the anterior lobe of the pituitary, he was able to check the effect of his transplants by observation of the total, or partial absence, of the characteristic symptoms of hypophysectomized animals. Having proved the effectiveness of such substitution therapy, he extended his observations to the results obtained by superimposing the transplants upon normal animals in various stages of development, thereby inducing a state of hyperstimulation.

Smith's work consisted in a series of daily anterior pituitary transplants, from mammalian donors, into immature female mice or rats. Such transplants induced with striking rapidity a series of changes involving all parts of the genital system. The vaginal introitus became established and permitted the insertion of a small spatula. The vaginal smear indicated an oestral condition and that ovulation would soon take place. If autopsy was performed, the uterus was greatly distended and hyperemic. Many follicles nearby, ready to rupture were seen on the surface of the ovary.

If autopsy was postponed for a day, there had been progress of the sex cycle, and the picture changed. The uterus was not distended, but rugose and muscular. The ovary contained numerous corpora lutea which had increased the size of the organ considerably above that of the pre-ovulation stage, and the vaginal smear revealed a post-oestral condition. The changes in general then may be summarized as the induction of precocious sexual maturity.

The most marked change was the greater size of the ovaries of animals, which had received the pituitary transplants in compari-

son to those found in control mates, which had received no transplants, but had matured normally. On the basis of relative percentage weights, the combined ovarian weights of treated animals were respectively 8, 9, 10, and 14 times that of untreated, or normally maturing controls. Thus in addition to producing a precocious sexual maturity, with pituitary gland transplants, an ovarian gigantism was produced. The enlargement of the ovaries was not due to any increase in size of follicles or corpora, but to an increase in number. Structurally no deviation from the normal was seen in either follicles or corpora.

Smith summarized these changes as follows:

The presence in the ovaries of the treated animals of a large number of approximately mature follicles or corpora, together with the relative absence of atretic follicles is correlated with the occurrence of very large numbers of tubal ova, found when autopsy is performed after ovulation. . . . The number of these ova exceeds very considerably the greatest number ever reported for these species.

These ova cannot be distinguished histologically from any normal ova. These changes which occur in the ovary constitute the anatomical picture of superovulation.

Changes in other organs may be briefly summarized as follows: The uterus shows a slight increase in weight, as compared with normally maturing controls, while histologically the two are identical. The only marked change therefore, is a precocious, but otherwise normal uterine maturity. Vaginal changes consist in establishment of precocious introitus and oestrus.

The significant fact in regard to the uterine and vaginal changes is that following total ovariectomy, pituitary transplants have no effect upon the uterus or vagina.

If, however, the ovarian hormone (follicular) alone is administered to these spayed animals, the changes noted above occur. Furthermore following ovariectomy pituitary transplants will not prevent the atrophy of uterus and vagina which normally occurs with castration.¹⁹

These observations indicate that at least one action of the hormone of the anterior lobe of the pituitary body is directly upon the ovary, and that the changes in the ovary vary according

to the age of the recipient, inducing in the immature mouse or rat precocious ovulation, and in the adult, superovulation. Such changes in the ovary as a result of increased amounts of the pituitary hormone have led to the designation of the pituitary by Zondek as the "motor of the ovary."

Not only does the anterior pituitary lobe exercise a direct control over the development and activity of the gonads, but it in turn is influenced by the genital organs. Comte³ and later others, have noted the hypertrophy of the hypophysis during pregnancy. Erdheim and Stumme⁵ demonstrated that a cell type characteristic of pregnancy arises from the chief or chromophobe cells.

The hypertrophy of the anterior hypophysis following total extirpation of the ovaries was first found in animals by Fichera⁸ and has since been repeatedly corroborated. Tandler and Gross²⁰ demonstrated an enlargement of the sella turcica of eunuchs by means of x-ray, and Rossle¹⁶ has shown that castration in humans results in a gross hypertrophy of the anterior hypophysis which histologically is due to an increase in eosinophilic cells, and the appearance of a much-debated type of cell, the so-called castration cell.

Recently Evans and Simpson⁷ have accumulated data which would seem to indicate that "the functional integrity of male and female germ cells is essential for the utilization of the anterior hypophyseal sex hormone."

We may therefore conclude that functional changes in the uterus and ovaries, such as those occurring during pregnancy, and probably in certain pathological complications or sequelae of pregnancy such as hydatid mole and chorioepithelioma should produce stimulus to the anterior lobe of the hypophysis, which in turn might be manifested by hyperactivity of this gland.

That such is the case has been demonstrated by Aschheim and Zondek¹ who have shown that the blood of the pregnant woman contains an abundance of ovarian hormone and of the pituitary hormones, and that they disappear on the second or third day of the puerperium. This would seem to indicate that they are a specific formation, at least in large and demonstrable

amounts, in pregnancy. Nature works with extreme over-production in everything pertaining to propagation, and the organism freely excretes what is not necessary. In the case of the hormones, the massive excretion takes place in the urine. These two hormones are excreted in the urine in a quantitatively different manner.

The amount of the ovarian hormone which is excreted in the urine during pregnancy rises gradually and slowly in the first eight weeks of pregnancy, and then very suddenly until it reaches its climax in the last two months. The amount of the pituitary hormone which is excreted in the urine rises steeply to its climax immediately after conception and remains at its climax from the second week to the middle of pregnancy. From then on it falls very slowly.¹

If then we have a means for demonstrating the presence of a particular hormone, and that hormone is excreted in the urine in demonstrable amounts during pregnancy only, then the demonstration of that particular hormone in the urine should be equivalent to the demonstration of pregnancy. And indeed it is upon the basis of such a demonstration that the Aschheim-Zondek test for pregnancy, as well as all modifications of the method of demonstration of the hormone of the anterior lobe of the hypophysis rests. Such tests should be designated hormonal tests for pregnancy, since the test rests on the demonstration of the hormone and not upon the demonstration of pregnancy. However, other tests for pregnancy have been devised, the technique of which rested on the demonstration of the ovarian hormone, that of Frank⁹ being the most outstanding example. So in order to avoid confusion it is necessary to refer to the test based upon the demonstration of the anterior pituitary secretion as the Aschheim-Zondek test. According to these authors¹ the diagnosis of pregnancy from the urine is an ancient practice of some 3,000 to 4,000 years standing, for it is described in an old Egyptian papyrus "that a woman may determine if she is pregnant by taking some earth and barley in a vessel and adding to it a little of her urine day by day. Should the barley grow, the woman is pregnant, but if the grain does not grow, then she will not bear a child."

The ovarian hormone is negligible in the early diagnosis of pregnancy because its excretion in the urine is not constant in the first eight weeks, and because it is also formed and excreted in functional disturbances of the ovary, and in various other diseases, as climatericum, hyperhormonal amenorrhea, tumors, and so forth.

The hormone of the anterior lobe of the pituitary body, (that is its massive production in the organism and excretion in the urine, up to a thousand times as much as normal) is specific of the earliest stage of pregnancy. Therefore, its demonstration in the urine is a means of early diagnosis.

The work of Aschheim and Zondek amply demonstrates that the diagnosis of pregnancy by means of this test is not only theoretical but practical. They have tested over a thousand specimens of urine with an accuracy of 98.6 per cent. Similar results have been reported from many other laboratories, so that it is perfectly evident that success in its utilization is not confined to the originators of the test. The details of the test have appeared in many papers, and we shall not repeat them here, other than to point out that the animals used were immature female white mice about three weeks of age and weighing between 5 and 8 gms. The injections of urine were made subcutaneously, and in six portions, three the first day and three the second day, and the ovaries were examined for sign of precocious maturity at the end of ninety-six hours. Five immature mice were used for each test.

We began working with this test in the fall of 1929, and soon became aware of a number of difficulties in the technique of the test rather than the end results. The first difficulty naturally was that of obtaining immature white female mice. We may point out that Aschheim and Zondek maintained a colony of 10,000 white mice as the source of their supply. The care of a colony sufficiently large to supply five immature female white mice at the proper stage for each test as needed, entails considerable expense and difficulty. In the second place the test requires about 100 hours for its completion.

Since the test finds its greatest value in differential diagnosis,

and actually makes the Clinical Pathologist a clinical consultant, its value would be greatly enhanced if this time period could be shortened. In hospitalized patients it is frequently not as practical to extend their hospitalization to ninety-six hours as to twenty-four hours, the economic factor being an important item as well as the clinical factor. And third, while the inspection of the mouse ovary in most cases reveals a clean cut positive or negative, in some cases it is difficult to interpret; and the use of five mice for each test, some of which are frequently positive while others are negative, would seem to point to the variation of absorption and utilization of the hormone even when present.

EXPERIMENTS

Encountering these difficulties in using mice in the test, we began experimenting with other animals in the hope of obtaining (1) an animal that could be used at any time, (2) an animal with larger ovaries in order that the end result might be more clean cut macroscopically, (3) an animal in which the end result might be obtained with greater rapidity. We began by working with immature and mature white rats, expecting to continue with the guinea pig and rabbit. We found the results with the immature white rat to be similar to those obtained with the white mice, and to give no improvement in general over the white mice.

It then occurred to us that if Graafian follicles were ripening constantly in immature animals and the hormone of the anterior pituitary was the stimulus needed to bring about ovulation and convert these follicles into corpora lutea, this change might be more readily and rapidly brought about if the hormone were administered to an animal that possessed mature follicles at all times but did not ovulate periodically. There are at least three such animals, namely the rabbit, the ferret, and the cat. In these animals ovulation does not occur until after copulation. In fact it has been demonstrated by Goichi¹³ that ovulation occurs within eighteen hours after copulation in the rabbit. Manifestly the rabbit was the most satisfactory laboratory animal of the three.

Since the hormone of the anterior lobe of the pituitary body is

physiologically liberated and distributed by the blood stream, we considered the intravenous administration of the urine logical. Our attention was directed to an article by Friedman¹⁰ in which he recorded the demonstration of the practical application of these principles. He reported a series of thirty-six rabbits which exactly checked with the subsequent clinical course in relation to pregnancy. Friedman's work, while primarily physiological in character, suggested the possibility of applying this test in routine clinical work. It consisted in comparing results obtained by the intraperitoneal and intravenous injection of the urine of pregnant women into non-pregnant does. The summary of Friedmans work is quoted.

1. It has been impossible to produce ovulation in the rabbit by transplantation of as many as 15 fresh rat hypophyses, or by the intraperitoneal injection of 24 fresh rat hypophyses. 2. Intraperitoneal injection of urine from pregnant women produces luteinization of the resulting corpora hemorrhagica in the rabbit ovary. 3. Single intravenous injection of urine from pregnant women provokes ovulation in the rabbit. 4. The samples of urine so far obtained from non-pregnant women have been utterly without effect on the rabbit ovary, either when injected intraperitoneally or when injected intravenously.

It appeared from this work, that the rabbit would supply the practical demands outlined above, if the reliability of the test equaled that of the Aschheim-Zondek test. With this in mind we began using the rabbit for the test, and whenever possible, comparing the results of the tests on rabbits with mice.

The animals should be adult female, non-pregnant rabbits weighing not less than four pounds. The urine from a suspected pregnant woman should be a catheterized morning specimen collected in a sterile container. It should be used as soon as possible, or if it must be retained for an hour or so, should be placed on ice at once, and so preserved until it is used. The amount of urine which we inject is from 5 to 12 cc. and the injection is made intravenously. The reason for the intravenous injection is that the end result is obtained much more rapidly by this method than by intraperitoneal injection. Thus, we may note the end results of the reaction in twenty-four hours after intravenous injection, whereas from four to six days are required

for the intraperitoneal and subcutaneous reactions. The positive reaction consists in the finding of corpora hemorrhagica or of corpora lutea in varying numbers usually in excess of those found in the natural course of ovulation; in other words, an ovulation induced by the substitution therapy.

The economy in the use of animals may be further extended if desired, by performing a laporatomy upon the animal and if negative, suturing the wound and using the same animal for a second test within a week or ten days. Animals, if the test is positive are not satisfactory for re-use, as luteinization and scarring of the ovary distorts it considerably for at least a month, and may result in a false interpretation of the results of a test.

Our experience with this test so far, is limited to a series of fifty cases, which have been checked by subsequent clinical history. Of these fifty cases the hormone diagnosis of pregnancy as revealed by subsequent history or operation has been supported in forty-nine cases. In one case the test was negative and at operation the patient presented the usual signs of pregnancy. The appendix was removed and the abdomen closed. A subsequent test on this patient was strongly positive. We do not know what was the cause of the failure in the first test, but if the urine was not mixed with some other patient's urine, then we must assume either a temporary absence of the hormone from the urine, or the presence of something either eliminated with the urine, or added to the urine after elimination which destroyed the hormone in that specimen. That the difficulty was not in the animal in this particular case is revealed by the fact that when subsequently injected with a known positive urine, there was a positive response. We would like to point out that from the pathological standpoint the investigation of failures with the utmost care is of extreme importance.

The earliest case that we have been able to check definitely is one in which the date of coitus was known. Twenty-one days later, the patient having missed her menstrual period meanwhile, this test for pregnancy was markedly positive. Subsequent observation confirmed the diagnosis.

Another case which proved interesting and at the same time

demonstrated the value of the test, occurred in a sanatorium for tuberculosis. The patient, suffering from advanced tuberculosis when admitted, had missed two menstrual periods. The physician in charge felt it was due to tuberculosis, while the obstetrician believed she was pregnant. The test was positive, and was later proved to be correct.

Gynecologists are not infrequently confronted by patients who have fibroids, become pregnant, and desire a hysterectomy for fibroids, concealing the part of their history which relates to pregnancy, in order that the pregnancy may be interrupted. We have had two cases of this kind, in which while the fibroids were present, the test proved positive for pregnancy, and the gynecologist refused to operate. At the last report both cases were progressing with pregnancy satisfactorily.

We have not had the opportunity of carrying out the test on a hydatidiform mole or a chorioepithelioma. However, Aschheim and Zondek report the value of such tests in these cases. The urine from these patients contains an abundance of the hormone of the anterior pituitary body, and the test is positive. This then provides a means of checking the progress of such a condition as well as a test for retained living placental tissue, and in all cases of miscarriage, abortion, etc., the test should be tried at frequent intervals, and when the test becomes negative, it seems justifiable to assume that there is no more living placental tissue present.

While the number of cases that we are reporting seems rather small in comparison to some of the series reported with mice as experimental animals, we might say that we have worked exclusively on the practical application of a demonstrated physiological fact. All of our cases have been those in which the practical question of clinical differential diagnosis has been involved. We have not run this series with the idea of producing a massive number of cases for statistical purposes. This was not possible with us because we could not secure a sufficiently large number of non-pregnant does to provide such a series. However, we have demonstrated to our own satisfaction, first, that by maintaining constantly a colony of twelve non-pregnant does, we have

sufficient animals to supply the ordinary clinical demands for the test; second, that in this test we seem to have about the same correct diagnostic percentage as in the Aschheim-Zondek test; third, that the intravenous method with rabbits greatly accelerates the time required for the test, and fourth, that in the adult female rabbit we have an animal that may be used at any time.

CONCLUSIONS

In conclusion we desire to emphasize the practical phase that this modification of the Aschheim-Zondek test brings to the Clinical Pathologist. These are (1) accessibility of the proper animal, (2) low cost in the care of the animals, (3) an animal that is available for the test at all times, (4) rapidity of obtaining the diagnosis, and (5) the ease with which the end results are interpreted.

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UNEXPECTED AUTOPSY FINDINGS IN UNEXPECTED DEATHS*

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Death is usually anticipated. Physiological death resulting from senility and the gradual wearing out of organs and tissues is probably an exceptional occurrence. The end usually comes as a culmination of some disease of whose ravages the clinician and the patient are aware. Sometimes it comes with suddenness as the result of an accident, of the imbibition of a poison, or of a sudden turn for the worse in an already existing disease, and sometimes it comes for no apparent reason. It is the unexpected cases of death to which reference is made in this paper.

When one hears of an unexpected death, one's mind usually turns to two, or at most three, of the systems of the body. One thinks first perhaps of the circulatory system, and secondarily of the nervous and respiratory systems, and as a matter of fact, the majority of cases of unexpected death may be referred to one of these. But in the course of autopsy experience one learns that there is a wide range, in lesions of these systems, and that the primary cause of the demise may occasionally originate in other systems and tissues, although the actual termination of life may be due to the effect upon one of the three systems previously mentioned. The cases reported have, with a few exceptions been selected from a series of about 1200 autopsies performed at the Hamilton General Hospital during the past ten years. Many of the more unusual observations have been revealed in autopsies done on cases, which have been the subjects of coroner's investigations, and where little or no previous clinical history was available. No doubt some of the so-called *unexpected* findings

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would have been *expected* had the cases been studied clinically before hand, but that is a condition which does not, as a rule, obtain in cases of unforeseen death.

The most common lesions of the heart which result in unexpected death are myocardial degeneration, coronary thrombosis or embolism, and infarction of the heart muscle. The following cases, in my experience at least, are unusual.

LESIONS OF THE CIRCULATORY SYSTEM

Extreme fatty degeneration of heart muscle

A young girl has contracted syphilis at the age of seventeen years. She had been treated with arsenicals in the out-patient department of the hospital, and after the second course of treatment had developed jaundice and for this reason had been admitted to the ward. The jaundice disappeared and the condition apparently improved. On the day before she was to have been discharged, she got out of bed to go to the toilet, as she had been doing for some days, and on the way collapsed and died in about fifteen minutes. At autopsy, the liver was found to be enlarged and fatty, and the heart weighed 350 grams and in appearance and on section was like putty. Sections showed an advanced degree of fatty degeneration of the myocardium. No other gross pathological condition was found sufficient to account for death. Apparently some undue exertion had caused interference with the conducting bundles and had caused the sudden demise. The comparative youth of the patient is the interesting factor.

Traumatic hematopericardium

A farm worker, aged twenty-four years took pleasure in teasing a lad aged fourteen years who was a summer visitor on the farm. In the course of an altercation, the boy picked up a pitchfork and hurled it from a distance of a few feet at the hired man. The man gave a cry of pain but did not seem to be seriously hurt. In a few minutes, however, he complained of feeling faint and a physician was called, who, on arrival a short while later found

him breathing his last. Physical examination showed a very small puncture wound between the second and third ribs on the right side with no bleeding. A coroner was notified who ordered an autopsy. Upon examination, it was found that one prong of the fork, which was small, had penetrated through the lung, through the upper part of the pericardium and had torn a hole in the aorta about 1 cm. in length, from which blood had been pumped until the pericardium was distended to a degree which caused cessation of the heart's action.

The same finding was observed in another case where the wound was caused by the blade of a jack-knife in a stabbing affray between a colored man and his paramour, the former being the victim.

Aortic stenosis

A man aged forty-five years was found dead in bed at his rooming house. No history of recent previous illness could be elicited. At autopsy, an hypertrophied heart weighing 450 grams and with a marked stenosis of the aortic valve was found. No other lesions not related to this were discovered. It is probable that in cases like this one the fibrosis of the myocardium which accompanies the hypertrophy may finally interfere with one or the other of the conducting bundles of the heart.

Congenital absence of ventricular septum

The usual congenital abnormalities found are patent foramen ovale, patent ductus arteriosus, and patent ductus venosus. In this case of a new-born infant which was poorly nourished and which lived only three days, there was a congenital absence of the ventricular septum which gave the child a three-chambered heart.

Coronary thrombosis at fourteen years

A young Polish girl, aged fourteen years, skipped 300 times at school in the morning and had a pain in the precordial region, which passed away. At noon she began her lunch, had a return of the pain, vomited, and died in five minutes. An autopsy was

ordered by the coroner. The aorta was found to show a marked degree of atheroma, some of the patches showing ulceration. The mouth of the left coronary artery was almost occluded by vegetations. The first 2 cm. of the coronary artery were blocked by a thrombus and the surrounding tissues in this area showed inflammatory change. On going into the history, it was found that the girl had had attacks of scarlet fever, measles, chicken pox, diphtheria and whooping cough, since the age of six. The interesting point about this case is the age.

LESIONS OF THE RESPIRATORY SYSTEM

Lobar pneumonia

A street cleaner, aged about sixty years, had been in the city employ for about five years. One day he collapsed on the street and was brought to the hospital in the police ambulance. He died about one hour after admission. At autopsy lobar pneumonia involving the whole of the right lung in the gray hepatization stage was found. The heart showed considerable myocardial degeneration. The man had been apparently carrying on his work while suffering from lobar pneumonia for several days.

Influenza pneumonia

A man aged about forty-five years, had been actively carrying on his work as a manager. One morning he felt chilly but carried on his work until lunch time. After lunch he became quite ill, finally going into coma and dying a few hours later. At autopsy both lungs were found to be involved by a hemorrhagic type of bronchopneumonia from which *Streptococcus* (hemolytic) was cultured. There was also some edema of the lungs. The man had apparently suffered from a fulminating attack of bronchopneumonia of the influenzal type.

LESIONS OF THE NERVOUS SYSTEM

Cerebellar tumor

A patient in the Hamilton General Hospital had been brought in for diagnosis with a tentative diagnosis of brain tumor. A

diagnostic lumbar puncture was performed and 12 to 15 cc. of clear spinal fluid was removed. The patient went into convulsions and died within an hour. At autopsy a glioma of the cerebellum was found which had been pressed down into the foramen magnum so as to leave the imprint of the foramen magnum on it. The disturbance of pressure brought about by lumbar puncture had apparently caused sudden death.

Hemorrhage into cerebral cyst

A workman, aged twenty-four years, on his way to work one morning bumped his head slightly on the top of the car when getting into it. In a few minutes he lapsed into unconsciousness and was taken to a doctor's office from where he was sent to the hospital dying shortly after admission. At autopsy there was found a large blood-filled cyst of the anterior part of the right cerebral hemisphere. This cyst was roughly 8 cm. by 6 cm. There was no evidence of neoplastic tissue in the walls of the cyst. Apparently the slight blow which left no mark on the skin was sufficient to cause a rupture of a vessel bordering on the cyst. The hemorrhage had burst into the right lateral ventricle.

Death in epilepsy

A gentleman was found in his apartment one morning dead with his face buried in his pillow. Apparently he had been well the night before. At autopsy there was marked congestion of the face and petechial hemorrhages scattered over the neck, arms, and chest. Internally there were the usual evidences of suffocation. It was hard to understand how an apparently healthy man of forty could suffocate in this manner. He had explained previous periods of illness by stating that he had had a kidney removed during the war and suffered from renal insufficiency. There was no evidence at autopsy of a kidney wound or operation; both kidneys were of normal size and appearance except for congestion. The history, secured later, showed that he had been suffering from epilepsy and used this story to explain periods of indisposition. He died from suffocation in an epileptic attack.

LESIONS OF THE ALIMENTARY SYSTEM

Appendiceal hemorrhage

A young woman, aged twenty-five years, was brought into the hospital, having been ill for three days and having passed blood from the bowel on the day before. She was very anemic. A diagnosis of typhoid fever was made but Widal reactions were negative. She died on the day following admission. At autopsy the appendix was thickened and ulcerated and imbedded in a mass of adhesions. Dissection showed that it contained a small black-headed pin about 2 cm. in length. Ulceration had taken place but without external rupture of the appendix. The lumen of the appendix was patent and had served as a drainage tube for blood coming from an eroded artery. The patient died of hemorrhage.

Infected Meckel's diverticulum

A young man, aged twenty-three years, was admitted to the hospital with a diagnosis of general peritonitis. A drain was inserted but he died on the third day after admission. At autopsy a small piece of impacted fecal material was found in a badly ulcerated Meckel's diverticulum and this appeared to be the starting point of the infection.

Traumatic rupture of spleen

A young soldier in Macedonia during the late war had had repeated attacks of malaria. He was thrown from his horse one day and went into a state of collapse from which he did not recover. He died the next day. At autopsy the spleen weighed 2000 grams and had ruptured from the fall.

LESIONS OF THE GENITO-URINARY SYSTEM

Unexpected death occasionally occurs from acute uremia. A boy, aged sixteen years, in coma was admitted from a boy's home. At the time no history of nephritis could be obtained. He died within forty-eight hours of acute uremia. At autopsy, contracted kidneys were discovered. Further inquiry elicited

the fact that the boy at the age of eight years had had a very mild attack of scarlet fever.

STATUS LYMPHATICUS

Every pathologist must carry in his mind this condition in cases of unexpected death in young people. The following case illustrates this condition:

A boy, aged twelve years, was in a boat with several older boys. An altercation arose and one of the boys struck at him with an oar. He fell overboard and never rose to the surface. The body was recovered some hours later. Autopsy showed no evidence of injury and absolutely none of the evidences of drowning. It did reveal, however, the classical anatomical evidence of status thymico-lymphaticus. The thymus weighed 45 grams. There was great hyperplasia of the lymphoid elements, especially of the mesenteric glands, and the lymphatics of the colon, with a relative hypoplasia of the heart and aorta. The boy had apparently succumbed to the shock occasioned by sudden fear.

VIOLENT DEATHS

Rupture of duodenum

A workman in a sand pit was crushed between the wall of the pit and the hub of a wagon. He was admitted to the hospital, and two days later showed symptoms of intestinal obstruction. No operation was performed and he died the next day. At autopsy there was no peritonitis but dissection of the duodenum showed it to have been torn across by being pinched against the vertebral column. The surrounding retroperitoneal tissues were inflammatory and to some extent infiltrated with duodenal contents. There was a small hematoma at the root of the mesentery.

Bullet in aorta

A workman was shot through the chest in a brawl. Post-mortem examination showed the bullet to have traversed the right side of the chest, struck the body of the fifth thoracic vertebra, and made a hole at this point in the aorta. It was found in the aorta at a point about 3 cm. above the bifurcation.

Acute pulmonary edema

In several accidents, death has been caused by the onset of edema of the lungs where the injuries seemed trivial. It is probably associated with nervous system shock.

No doubt many pathologists have seen cases similar to these reported but one should always remember that sudden death can be brought about by a wide variety of causes arising in almost any one of the organs or systems of the body. Attention directed to unusual cases of this kind is always of benefit.

ADDITIONAL OBSERVATIONS ON ISOLATING TUBERCLE BACILLI

THE OXALIC ACID REAGENT FOR PRIMARY CULTURE*

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In a study reported last year (Corper and Uyei¹) as part of a plan to improve and elaborate upon practical methods for the certified diagnosis of tuberculosis it was found that a 5 per cent oxalic acid reagent might possibly be substituted with advantage for the 6 per cent sulphuric acid reagent originally recommended in the new sulphuric acid-crystal violet potato medium method for isolating tubercle bacilli from tuberculous materials (Corper and Uyei^{2,3,4}).

In the report it was shown that the 5 per cent oxalic acid reagent was superior to the six per cent sulphuric acid reagent for isolating tubercle bacilli from tuberculous sputums in that a greater percentage of the total tubes planted yielded positive cultures of tubercle bacilli, this being accounted for by the fact that the oxalic acid reagent was less toxic to tubercle bacilli as determined by bacteriostatic tests and also by the fact that the oxalic acid reagent possessed a greater germicidal action for the contaminating organisms found in sputum. Although the foregoing results obtained with tuberculous sputums and the results of the bacteriostatic experiments were in favor of the oxalic acid reagent over the sulphuric acid reagent, we still hesitated to recommend the oxalic acid reagent at that time to replace the sulphuric acid reagent in the new method of cultivating tubercle bacilli until

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† Dr. H. J. Corper was awarded the Ward Burdick Medal for his work on the cultivation of tubercle bacilli.

more data with a greater variety of materials and specimens had been obtained.

It is the purpose of this contribution to report such studies with urines and contaminated tuberculous animal tissues which corroborates the previous findings with sputums in indicating that the 5 per cent oxalic acid reagent can be used to replace the 6 per cent sulphuric acid reagent in the sulphuric acid-crystal violet potato cylinder method for cultivating tubercle bacilli from tuberculous materials if desired.

TABLE 1
A COMPARISON OF THE 5 PER CENT OXALIC ACID REAGENT WITH THE 6 PER CENT
SULPHURIC ACID REAGENT FOR ISOLATING TUBERCLE
BACILLI FROM URINE

| REAGENT USED FOR DESTROYING CONTAMINATORS | NUMBER OF TUBES OF POTATO MEDIUM PLANTED | NUMBER OF TUBES THAT BECAME CONTAMI- NATED | NUMBER OF TUBES IN WHICH TUBERCLE BACILLI WERE ISOLATED | PERCENT- AGE CONTAMI- NATIONS | PERCENT- AGE ISOLATIONS |
|--|--|---|---|--|-------------------------------|
| 5 per cent oxalic acid..... | 144* | 52 | 37 | 36 | 26 |
| 6 per cent sulphuric acid..... | 144 | 59 | 36 | 41 | 25 |

* Twenty-four specimens of urine were tested and each urine planted on six tubes of glycerol water-crystal violet-potato cylinder medium.

I. A COMPARISON OF OXALIC ACID WITH SULPHURIC ACID FOR THE ISOLATION OF TUBERCLE BACILLI FROM TUBERCULOUS URINES

In this experiment, twenty-four specimens of urine were used. These urines were obtained from patients suspected of having renal tuberculosis or known to have renal tuberculosis. The urines were tested using the technic previously described by us for isolating tubercle bacilli from tuberculous materials; as reagents for destroying contaminants either the 6 per cent sulphuric acid reagent or the 5 per cent oxalic acid reagent were used in comparative tests. After treating the sediment of urine specimens with an equal volume (one cubic centimeter) of the acid reagent and diluting with sterile saline solution after thirty minutes incubation with occasional shaking, the washed sediment was planted on six tubes of glycerol crystal violet potato medium for

each specimen of urine and for each of the acid reagents tested. Thus one hundred and forty-four tubes of medium were planted from the twenty-four urine specimens for each reagent tested. The planted potato culture tubes were incubated at 37°C. and at weekly intervals were examined and the amount of growth found was recorded. All positive macroscopic findings were checked by examining smears stained by the Ziehl-Neelsen method for the presence of acid-fast bacilli. The results of these findings are recorded in table 1.

The findings recorded in table 1 indicate that there is a slight superiority of the 5 per cent oxalic acid reagent over the 6 per cent sulphuric acid reagent in that the use of the former with urines results in a smaller percentage of contaminations and a slightly greater percentage of isolations of tubercle bacilli. This agrees well with the results recorded previously for tuberculous sputums in which the 5 per cent oxalic acid reagent yielded 88 per cent isolations of tubercle bacilli and 15 per cent contaminations, as compared to 78 per cent isolations of tubercle bacilli and 28 per cent contaminations with the 6 per cent sulphuric acid reagent.

II. THE ISOLATION OF TUBERCLE BACILLI FROM INFECTED TISSUES WITH THE OXALIC ACID REAGENT OR THE SULPHURIC ACID REAGENT

In this experiment tissues were obtained from four dogs and four rabbits previously given intravenous injections of different amounts of fine suspensions of virulent human tubercle bacilli (Gluckson). After a lapse of four to fourteen days after the injection of the tubercle bacilli, the animals were killed by electrocution and specimens of five different tissues, namely, lung, liver, spleen, kidney, and bone marrow, from each animal were used for the isolation of the tubercle bacilli from the tissues. A specimen of the tissue usually a piece about the size of a pea was finely ground up in a porcelain mortar using about three to five cubic centimeters of 0.9 per cent saline solution to obtain a good mixture. About 1 cc. of the tissue suspension in the saline solution, as free as possible from large lumps, was submitted to the

routine isolation technique for tubercle bacilli using either an equal volume (1 cc.) of the 5 per cent oxalic acid reagent or of the 6 per cent sulphuric acid reagent and after incubation and dilution

TABLE 2

A COMPARISON OF THE OXALIC ACID REAGENT WITH THE SULPHURIC ACID REAGENT FOR THE ISOLATION OF TUBERCLE BACILLI FROM INFECTED TISSUES OF THE DOG AND RABBIT

| ANIMAL USED FOR INTRAVENOUS INFECTION | WEIGHT OF ANIMAL | AMOUNT OF BACILLI INJECTED PER POUND BODY WEIGHT | REAGENT | TISSUES FROM WHICH TUBERCLE BACILLI WERE ISOLATED AND NUMBER OF CULTURE TUBES (OF A TOTAL OF FIVE USED) WHICH PROVED POSITIVE FOR TUBERCLE BACILLI | | | | |
|---------------------------------------|------------------|--|-------------|--|-------|--------|--------|-------------|
| | | | | Lung | Liver | Spleen | Kidney | Bone marrow |
| Dog | pounds | mgm. | | | | | | |
| | 30 | 1.0 | { Oxalic | 4 | 5 | 5 | 5 | 5 |
| | | | { Sulphuric | 5 | 4 | 5 | 5 | 5 |
| | 27 | 0.01 | { Oxalic | 4 | 5 | 4 | 4 | 0 |
| | | | { Sulphuric | 3 | 5 | 5 | 5 | 0 |
| | 18 | 0.000,1 | { Oxalic | 3 | 2 | —* | 0 | 1 |
| | | | { Sulphuric | 2 | 5 | —* | 0 | 1 |
| | 28 | 0.000,001 | { Oxalic | 2 | 4 | 2 | 1 | 4 |
| Rabbit | | | { Sulphuric | 1 | 5 | 2 | 0 | 4 |
| | 9 | 1.0 | { Oxalic | 3 | 5 | 5 | 5 | 5 |
| | | | { Sulphuric | 3 | 5 | 5 | 5 | 5 |
| | 7.5 | 0.01 | { Oxalic | 4 | 5 | 5 | 5 | 5 |
| | | | { Sulphuric | 1 | 5 | 4 | 5 | 4 |
| | 8 | 0.000,1 | { Oxalic | 4 | 3 | 4 | 0 | 5 |
| | | | { Sulphuric | 4 | 2 | 4 | 0 | 5 |
| | 9.3 | 0.000,001 | { Oxalic | 1 | 3 | 4 | 3 | 4 |
| | | | { Sulphuric | 2 | 2 | 3 | 4 | 4 |

* The spleen from this dog was not studied.

with saline solution, the centrifugate planted upon the glycerol water-crystal violet potato medium and incubated. The quantity of tubercle bacilli injected intravenously into the dogs or

rabbits varied from 1.0 milligram to 0.000,001 mgm. per pound body weight of the animal that was injected.

The findings in this experiment are recorded in table 2 as the number of potato culture tubes found positive from each specimen of tissue taken from the different organs, following the use of either the oxalic acid or sulphuric acid reagent; a maximum of five tubes having been planted for each specimen of tissue taken and submitted to examination for tubercle bacilli.

The results of the study with infected animal tissues recorded in detail in table 2 become more evident when the findings are summarized as given in table 3 and when the number and percentage of contaminations are also noted.

TABLE 3
SUMMARY OF COMPARISON OF THE OXALIC ACID REAGENT WITH THE SULPHURIC ACID REAGENT FOR THE ISOLATION OF TUBERCLE BACILLI FROM TUBERCULOUS ANIMAL TISSUES

| REAGENT USED IN EQUAL VOLUME | TOTAL NUMBER OF POTATO CUL- TURE TUBES USED FOR 39 TISSUE SPECI- MENS TESTED | NUMBER OF TUBES POSITIVE FOR TUBERCLE BACILLI | NUMBER OF TUBES CONTAMI- NATED | PER CENT ISOLATION OF TUBERCLE BACILLI | PER CENT CONTAMI- NATIONS |
|--------------------------------|--|--|---|--|---------------------------------|
| 5 per cent oxalic acid. | 195 | 138 | 26 | 71 | 13.8 |
| 6 per cent sulphuric acid. ... | 195 | 134 | 39 | 69 | 20.0 |

It is evident from the results recorded in tables 2 and 3 that the 5 per cent oxalic acid reagent possesses a slight but definite advantage over the 6 per cent sulphuric acid reagent for isolating tubercle bacilli from infected tissues. The results are not so evident when the number of positive findings for tubercle bacilli are considered from the standpoint of the tissues tested as when the number of positive culture tubes are noted. It is especially significant that the number of contaminated tubes was higher with the sulphuric acid reagent than with the oxalic acid reagent. These findings with tissues corroborate those previously recorded with sputums and bear out the findings with urines in indicating that the 5 per cent oxalic acid reagent possesses a decided, though slight, advantage over the 6 per cent sulphuric acid reagent for

destroying contaminators and increasing the number of positive cultures obtained from the use of the acid glycerol water-crystal violet potato cylinder method for detecting tubercle bacilli in suspected tuberculous materials.

III. THE ISOLATION OF BOVINE TUBERCLE BACILLI

In our earlier experiments performed to perfect the method of isolating tubercle bacilli from tuberculous materials when present in small numbers the magnitude of the problem prevented an exhaustive study with bovine tubercle bacilli and most of the tests with this organism were restricted to the use of a number of laboratory strains which had been under artificial cultivation for some years. Although it was noted at that time that the potato medium supported the growth of small numbers of bovine tubercle bacilli slightly less efficiently than it did human tubercle bacilli, the differences were not especially striking and particularly so since the potato medium was found to be the best nutrient medium, of all the mediums tested, for supporting the growth of small numbers of bovine tubercle bacilli. Shortly after the glycerol water-crystal violet-potato method was described by us Dr. William H. Feldman⁶ found in trying to use this method for isolating bovine tubercle bacilli that it did not give as gratifying results as when isolating human tubercle bacilli and asked us to corroborate his findings and if possible find out the reason for this difference in behavior of the bovine and human bacilli. Accordingly strictly bovine specimens were immediately obtained from the government inspectors* examining cattle at the Denver Packing Houses.

In this experiment was included an examination of sixteen specimens taken from various organs in definitely tuberculous cattle as they came from the Denver slaughter houses on the day of inspection immediately after killing the cattle. The technique followed for isolation was identical to that previously reported elsewhere for the oxalic or sulphuric acid glycerol water-crystal

* We are grateful to Dr. T. A. Shipley and his assistants of the Meat Inspection Department of the United States Bureau of Animal Industry for their courtesy in supplying pathological specimens.

violet-potato cylinder medium method. Two reagents, namely 6 per cent sulphuric acid or 5 per cent oxalic acid were used for preliminary sterilizing to remove the contaminators. Six culture tubes containing glycerol water-crystal violet-potato cylinder medium were used for planting from each specimen of tissue, so that in all 96 tubes were used for each acid reagent. In addition to testing by the culture method, two guinea pigs were given the ground tissue material by subcutaneous injection in order to compare the efficiency of the culture method with the guinea pig method for the detection of bovine tubercle bacilli in bovine material.

Out of sixteen tissue specimens used, which included lung, liver, bronchial, mediastinal and mesenteric glands, seven specimens proved positive by the sulphuric acid reagent method and nine specimens proved positive by the oxalic acid reagent method, while all the specimens were positive by guinea pig inoculation. On the basis of the culture tubes planted, the sulphuric acid reagent method revealed twenty tubes out of ninety-six or 21 per cent positive isolations, while the oxalic acid reagent revealed twenty-four tubes out of ninety-six or 25 per cent positive isolations.

It is natural that the question of the cause of these inferior results with the culture method should merit elucidation in order to attempt to remedy the deficiency if possible and better adapt the culture method to include the detection of bovine bacilli. Naturally the solution resolves itself to determining whether the poor results are due to the toxicity of the reagents used to destroy the contaminators or whether the deficiency of the medium is accountable, the latter being suggested as a result of the earlier studies with laboratory strains of bovine bacilli.

In order to determine whether the toxicity of the acid reagents was accountable, the effect of 6 per cent sulphuric acid, 5 per cent oxalic acid and 3 per cent acetic acid on freshly isolated bovine tubercle bacilli was studied. For this purpose one cubic centimeter of a finely divided suspension of recently isolated bovine tubercle bacilli* containing one milligram, 0.01 or 0.0001 mgm. were mixed

* Some of the strains used in this and subsequent experiments were obtained from Dr. H. C. Sweany of the City of Chicago Municipal Tuberculosis Sanitarium, and from Dr. Wm. H. Feldman of the Mayo Foundation.

with one cubic centimeter of the reagents above being tested. After incubation of thirty minutes at 37°C. the mixture was diluted to ten cubic centimeters with sterile saline solution, centrifugated, decanted and the residual material was planted on tubes of the glycerol water-crystal violet-potato cylinder medium. The growth obtained at incubator temperature (37°C.) was compared with the growth obtained in control culture tubes in which the tubercle bacilli were planted after treatment with 0.9 per cent saline solution alone without the use of the acid reagents.

The findings in this experiment revealed no difference in the growth of bacilli on the control tubes as compared to the experimental tubes in which the bacilli had received the preliminary treatment with the oxalic or sulphuric acid reagents, indicating that these reagents are practically innocuous to the recently isolated bovine tubercle bacilli in the concentrations and manner tested which is that outlined in the oxalic or sulphuric acid glycerol water-crystal violet-potato cylinder method. This lack of harmful effect of the acids upon the bacilli in concentrations of an equal volume of 5 per cent oxalic acid or 6 per cent sulphuric acid was also confirmed by treating the tuberculous bovine specimens with either of these reagents and injecting the saline washed sediment into guinea pigs and comparing the findings with those obtained by inoculating guinea pigs in similar manner with the untreated specimen of tuberculous tissues. The tuberculosis developed in the controls and in the test guinea pigs to a like extent. These findings would therefore appear not to incriminate the oxalic acid reagent or the sulphuric acid reagent in accounting for the unsatisfactory results obtained in isolating bovine tubercle bacilli from fresh bovine tuberculous materials. There remains, therefore, the problem of determining whether the deficiency of the medium as a nutrient for small numbers of bovine tubercle bacilli can account for the unsatisfactory results obtained. This experiment was rather extensively pursued and can be divided into two parts; one (*a*) to note whether glycerol was detrimental and what concentrations if any could be used for growing small numbers of bovine tubercle bacilli and the other (*b*) to determine the comparative nutrient value of various mediums for growing bovine tubercle bacilli.

a. Determination of the optimum concentration of glycerol in the medium essential to growing the bovine tubercle bacillus

The optimum concentration of glycerol for growing human strains of tubercle bacilli when the latter are present in small numbers was previously found to be about 2 to 3 per cent concentration in the potato nutrient medium. The concentration of glycerol used in the crystal violet potato cylinder medium was so adjusted that the final concentration (using 6 per cent glycerol water) after introducing the planting fluid, etc. was 2 to 3 per cent.

The optimum concentration of glycerol for the growth of laboratory strains (several years of artificial cultivation) of bovine tubercle bacilli was previously (Corper and Uyei⁴) found to be about 2 per cent in potato broth agar medium. Since there is a possibility of change in glycerophilic property of tubercle bacilli, especially during long artificial cultivation in the laboratory, the optimal glycerol concentration in the medium for the growth of small numbers of recently isolated strains of bovine tubercle bacilli was also determined.

In this experiment one cubic centimeter of pure glycerol varying in concentration from 0.0 to 10 per cent in distilled water was used in the crystal violet potato cylinder medium, and on this medium was planted varying amounts of a number of recently isolated bovine strains of tubercle bacilli in fine suspensions.

It was surprising to note that all the recently isolated strains of bovine tubercle bacilli tested grew equally well in the potato mediums regardless of the amounts of glycerol added from 0.0 to 10 per cent showing that a wide range of glycerol can be used and that this factor is not pertinent for the isolation of small numbers of bovine tubercle bacilli on the potato cylinder medium. In order to further confirm this point experiments are now in progress to determine the effect of glycerol in the potato medium on the isolation of bovine tubercle bacilli from infected tissues.

b. A comparative study of various mediums as nutrients for the growth of bovine tubercle bacilli

A study of various mediums as nutrients for small numbers of recently isolated bovine tubercle bacilli has not advanced far

enough to make a definite report and therefore only the general plan of this phase of the study will be submitted, reserving the report of the results obtained for a subsequent article.

Among the mediums in contemplation for use and already under test for determining their nutrient value for growing small numbers of recently isolated strains of bovine tubercle bacilli and for isolating small numbers of bacilli from bovine tuberculous materials are included glycerol broth-gentian violet egg medium (Petroff), glycerol broth egg medium (Dorset), Evanoff-Sweany's⁵ cream medium, glycerol water-crystal violet-potato medium, plain sweet potato cylinder medium, glycerol water sweet potato cylinder medium, Jerusalem artichoke cylinder medium (with and without glycerol), potato milk medium, potato cream medium and others.

It is hoped thus by studying the nutritive value of various new substances, as well as suggestive combinations, to increase the culture efficiency especially for determining the presence of small numbers of tubercle bacilli in bovine tuberculous materials and finally if possible to devise a simple single medium to serve for both human and bovine tuberculous materials.

SUMMARY

In verification of previous findings reported for sputums it was found that a 5 per cent oxalic acid reagent, for destroying contaminators in the culture method for the diagnosis of tuberculosis, also proved slightly superior to a 6 per cent sulphuric acid reagent with specimens of urines and infected animal tissues. The superiority of the oxalic acid reagent expressed itself in a slightly higher percentage of positive culture tube findings in addition to a lower percentage of tubes contaminated and a lesser detrimental effect in comparative toxicity tests with suspensions of tubercle bacilli.

The use of the oxalic or sulphuric acid glycerol water crystal-violet potato cylinder method for detecting the presence of bovine tubercle bacilli in tissues was less satisfactory than for detecting human bacilli. The explanation for this resides in the inadequacy of the potato medium and not in the oxalic or sulphuric acid reagents used for destroying contaminators nor in the glycerol used

as part of the glycerol water crystal-violet potato cylinder medium. The potato medium has thus far proved to be the best medium, of the mediums tested, for growing small numbers of bovine tubercle bacilli and investigations are now in progress to find a more suitable nutrient for the bovine tubercle bacilli present in bovine tuberculous tissues.

Appreciation is due L. D. Miller and Margaret Uyei for assisting with the technical phases of this study.

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THE DIRECT CALCULATION OF THE VOLUME AND HEMOGLOBIN CONTENT OF THE ERYTHROCYTE*

A COMPARISON WITH COLOR INDEX, VOLUME INDEX AND SATURATION INDEX DETERMINATIONS

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Knowledge of physical variations in the erythrocyte which take place in the various anemias has been much hampered by the inaccuracy and inadequacy of hematologic technic. These defects until recently have passed unchallenged and even today the gross errors of the commonly used methods of estimation of hemoglobin and the wide variation in the gram-equivalent accepted as 100 per cent or "normal" for the various hemoglobino-meters is by no means generally appreciated. Advances in our knowledge have been further impeded by a lack of sufficiently well founded standards of the normal. It is only in the past few years that an attempt has been made to supply accurate standards for the normal red cell count, hemoglobin and volume of packed red cells ^{9,10,13,15,17,18}.

The color index affords a good example of the confusion and inaccuracy which is quite common. This index is meant to express in a given case the relative amounts of hemoglobin and erythrocytes in proportion to the normal, or, what is the same, the average hemoglobin content of the erythrocytes of the sample in proportion to the hemoglobin content of the erythrocytes of normal blood. For this index to be of any value it is essential that the technic of the hemoglobin and erythrocyte determinations which enter into its calculation be accurate and, furthermore,

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† Work done in Department of Medicine, Tulane University.

it is necessary that correct standards for normal be employed. Generally speaking neither of these requisites is met in the every day color index determination. The hemoglobinometer employed is frequently inaccurate and furthermore, 100 per cent or normal, as given by the various instruments, represents a wide range of values which vary from 13.8 grams of hemoglobin per 100 cc. of blood on the Dare instrument to 17.2 grams on the Sahli. The marked discrepancy in the value accepted as 100 per cent reflects the inaccurate foundation on which our conception of the normal has been based.

Further error arises from the fact that recently made accurate erythrocyte counts in healthy individuals^{13,17,18} indicate that the value which has been assumed to be normal or 100 per cent in the calculation of color index, namely five million erythrocytes, is not correct, the average erythrocyte count in normal men being distinctly greater than this figure. Again, the fact that the "normal" appreciably differs in the two sexes, is another obvious source of error.

The term "color index" is in fact an unfortunate choice and one of the most important arguments which can be brought against this index is the erroneous conception to which the term has given rise. Because in pernicious anemia the color index is greater than 1 whereas in the "secondary" anemias this index is low, there is a general impression that in the former disease the erythrocyte is supersaturated with hemoglobin, whereas in the "secondary" anemias the reverse is found. Color index, however, does not measure the hemoglobin saturation of cells, but rather their hemoglobin content⁶. Direct calculations of the volume and hemoglobin content of the erythrocyte indicate that in anemia one of the essential changes is an alteration in the average size of the erythrocytes. Increases or decreases in the amount of hemoglobin contained in the cells are, with the exception of certain types of anemia, proportional to alterations in the size of the cells^{18,20,14,19}. It should perhaps be pointed out that direct calculations of the volume and hemoglobin content of erythrocytes, as well as the various indices, refer only to mean values and give no information concerning the individual cells. Bearing

this in mind it can be said that supersaturation does not occur in pernicious anemia^{6,20}. In fact, it is probably true that the average erythrocyte in disease never contains more hemoglobin per unit volume than is found in the normal corpuscle. The erroneous conception regarding the hemoglobin content of the erythrocytes in the anemias may be directly attributed to the use of such a vague and inaccurate term as "color index."

The volume index of Capps,³ and the saturation index of Haden⁶ suffer from imperfections similar to those of the color index. These defects cause me to favor the direct calculation of

TABLE 1
RELATION BETWEEN CORPUSCULAR CONSTANTS AND THE INDICES

| | | | |
|------------------|---|---|--|
| Color index | $= \frac{\left\{ \begin{array}{l} \text{Hemoglobin} \\ \text{per cent} \end{array} \right\}}{\left\{ \begin{array}{l} \text{Erythrocytes} \\ \text{per cent} \end{array} \right\}}$ | Mean corpuscular hemoglobin (in micromicrograms) | $= \frac{\left\{ \begin{array}{l} \text{Hemoglobin} \\ \text{(gm. per 1000 cc.)} \end{array} \right\}}{\left\{ \begin{array}{l} \text{Erythrocytes} \\ \text{(millions per cu. mm.)} \end{array} \right\}}$ |
| Volume index | $= \frac{\left\{ \begin{array}{l} \text{Volume packed} \\ \text{erythrocytes} \\ \text{per cent} \end{array} \right\}}{\left\{ \begin{array}{l} \text{Erythrocytes} \\ \text{per cent} \end{array} \right\}}$ | Mean corpuscular volume (in cubic microns) | $= \frac{\left\{ \begin{array}{l} \text{Volume packed} \\ \text{erythrocytes} \\ \text{(cc. per 1000 cc.)} \end{array} \right\}}{\left\{ \begin{array}{l} \text{Erythrocytes} \\ \text{(millions per cu. mm.)} \end{array} \right\}}$ |
| Saturation index | $= \frac{\left\{ \begin{array}{l} \text{Hemoglobin} \\ \text{per cent} \end{array} \right\}}{\left\{ \begin{array}{l} \text{Volume packed} \\ \text{erythrocytes} \\ \text{per cent} \end{array} \right\}}$ | Mean corpuscular hemoglobin concentration (in per cent) | $= \frac{\left\{ \begin{array}{l} \text{Hemoglobin} \\ \text{(gm. per 100 cc.)} \times 100 \end{array} \right\}}{\left\{ \begin{array}{l} \text{Volume packed} \\ \text{erythrocytes} \\ \text{(cc. per 100 cc.)} \end{array} \right\}}$ |

the volume and hemoglobin content of the red corpuscle. As pointed out by Haden⁶ from information concerning the number of erythrocytes and the amount of hemoglobin in a given sample of blood, the actual amount of hemoglobin in the average erythrocyte may be calculated by simply dividing the amount of hemoglobin per unit volume of blood by the number of erythrocytes in the same volume. Similarly the average volume of the red cells of any sample of blood may be calculated by dividing the volume of packed red cells per unit volume of blood by the number of red cells per unit of volume^{6,14}. The concentration of hemoglobin in the average cell of the sample of blood is calculated in an equally simple manner by dividing the amount of hemoglobin by

the volume of packed erythrocytes per unit of volume. These direct calculations of the volume and hemoglobin content of the erythrocyte are simple and clear as to their import. They afford a much clearer and more accurate conception of the physical state of the erythrocyte in health and the alterations associated with disease than can be gained by the consideration of the indices. The direct calculations express the same relationship between hemoglobin, volume of packed erythrocytes, and number of erythrocytes as do the indices (Table 1), except that the latter express this relationship in proportion to a supposed normal. As has already been pointed out, the values taken as normal are incorrect and besides, since a single value is taken as the equivalent of 100 per cent, the normal inter-individual variation in hemoglobin, number of erythrocytes and volume of packed erythrocytes is disregarded. I believe, therefore, that this expression in relation to "normal" may well be sacrificed for the sake of greater clearness and accuracy.

The desirability of the direct calculation of the hemoglobin content of the erythrocyte is further supported by the growing tendency to report hemoglobin directly in grams per 100 cc. of blood. This manner of reporting hemoglobin has been proposed in order to avoid the confusion arising from the use of a large number of different values as the equivalent of 100 per cent and is meeting with more and more favor both in the laboratory and at the bedside. In the calculation of color index from data concerning amount of hemoglobin, reported in grams, it is first necessary to convert the hemoglobin to terms of percentage, a step which is unnecessary when direct calculations are made.

In the present paper, simple methods for the calculation of the volume and hemoglobin content of the erythrocyte will be given and normal values for men and women based on accurate blood determinations which I^{13,15,17} have carried out, as well as on blood determinations reported by other investigators will be presented. Details of the methods in the determination of number of erythrocytes, amount of hemoglobin and volume of packed erythrocytes, as well as an analysis of their accuracy, are fully elaborated in earlier papers and need not be repeated here.

In the choice of names for the corpuscular constants discussed in this paper the attempt has been made to devise terms which are simple and yet descriptive. It is particularly difficult to find a suitable term to denote the volume of the individual red corpuscle since confusion so readily occurs with the term sometimes used to refer to the volume of packed red cells, namely, "cell-volume." The term "individual cell volume" is used by Haden⁷. This is still somewhat confusing. The terms which I propose, namely mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, are presented because I believe they best fulfil the requisites of simplicity, clarity and uniformity.

MEAN CORPUSCULAR VOLUME

The volume of the average erythrocyte, or "mean corpuscular volume," may be calculated for any given sample of blood by dividing the volume of packed erythrocytes, expressed as cubic centimeters of red cells per 1000 cc. of blood, by the number of erythrocytes expressed as millions per cubic millimeter. The result expresses mean corpuscular volume in cubic microns.

The volume of packed red cells may be determined by centrifugalizing a measured quantity of blood in a suitable hematocrit¹⁶. This instrument, once so popular, has not received the general usage in the clinical laboratory which it merits. Its obvious disadvantage is the fact that relatively expensive mechanical means are required to secure adequate centrifugalization. This fact, coupled with a general lack of appreciation of the valuable and accurate information which the instrument affords, probably explains why the popularity of the hematocrit was so short lived. This is indeed unfortunate because with reasonable care in technic the determination of relative cell volume by the hematocrit method is very accurate. In the blood determinations which I^{13,15,17} reported, in spite of the special attention paid to accuracy, analysis of the methods employed showed that the probable error of the erythrocyte counts was 2 per cent and that of the hemoglobin determinations possibly even as high as 10 or 12 per cent, whereas the probable error of the hematocrit determinations was

0.5 per cent. Since the information derived from hematocrit determinations is to a large extent similar to that afforded by the determination of hemoglobin, in the face of the prevailing inaccurate methods of hemoglobin determination, the advantages of the hematocrit greatly outweigh any possible disadvantages.

It has been stated that it is very doubtful whether any accurate measure of absolute cell volume can be obtained by hematocrit methods. Thus, Price-Jones¹¹ considers that the tightness of packing of the cells cannot be assumed to be uniform from one lot to another. In reply to these objections it may be said that uniformity of packing can be attained by centrifugalizing the cells until no further decrease in total cell volume takes place. This is usually attained by centrifugalizing the sample for fifteen and almost always for thirty minutes at 3000 revolutions per minute. Only occasionally is more time required for complete packing. If, after an initial centrifugalization for thirty minutes, readings are made at intervals of five minutes until no further change in volume of packed cells is observed, even this slight error can be eliminated.

It might be expected that by centrifugalization all fluid is forced out of the cell and only hemoglobin and plasma are left, thus making it impossible to obtain a true estimate of the size of the cells. Some experiments of Campbell² may be mentioned in this connection. This investigator mixed measured amounts of packed red cells with equal volumes of 0.6 per cent and 0.9 per cent saline solutions. The former swelled but hemolysis did not occur. After permitting equilibrium to take place between cells and saline, the specimens were stirred up and centrifugalized again. Repeated experiments showed that the cells in the weaker saline solutions remained proportionately larger than the cells in 0.9 per cent saline even after centrifugalization.

Jorgensen and Warburg⁸ considered that the volume of the blood corpuscles is determined by osmotic forces so great that the force with which they are centrifugalized down is negligible compared with them. They add, further, that the volume of cells as determined by refractometric and viscosimetric methods agrees with the results determined by the hematocrit method.

TABLE 2
THE VOLUME AND HEMOGLOBIN CONTENT OF THE ERYTHROCYTE; FIFTY NORMAL
YOUNG WOMEN

| SUBJECT | AGE | CORPUSCULAR VOLUME | CORPUSCULAR HEMOGLOBIN | CORPUSCULAR HEMOGLOBIN CONCENTRATION |
|---------|-----|-----------------------|---------------------------|--|
| | | <i>cu. microns</i> | <i>γγ</i> | <i>per cent</i> |
| 1 | 30 | 70.9 | 27.0 | 38.8 |
| 2 | 28 | 80.8 | 27.4 | 34.0 |
| 3 | 18 | 76.6 | 27.4 | 35.8 |
| 4 | 20 | 74.2 | 27.9 | 37.6 |
| 5 | 17 | 77.6 | 30.0 | 38.7 |
| 6 | 21 | 76.6 | 26.4 | 34.5 |
| 7 | 18 | 78.0 | 30.4 | 39.0 |
| 8 | 24 | 82.9 | 29.0 | 35.0 |
| 9 | 20 | 73.7 | 27.0 | 36.7 |
| 10 | 20 | 75.1 | 27.0 | 35.9 |
| 11 | 19 | 84.2 | 28.8 | 34.1 |
| 12 | 20 | 76.5 | 27.0 | 35.2 |
| 13 | 19 | 75.3 | 26.8 | 35.6 |
| 14 | 20 | 79.4 | 25.8 | 32.6 |
| 15 | 19 | 73.8 | 26.6 | 36.1 |
| 16 | 20 | 85.7 | 27.1 | 37.3 |
| 17 | 20 | 81.6 | 28.8 | 35.4 |
| 18 | 20 | 80.0 | 26.3 | 32.9 |
| 19 | 18 | 81.3 | 27.8 | 33.6 |
| 20 | 23 | 82.9 | 30.4 | 36.6 |
| 21 | 20 | 82.1 | 28.0 | 34.4 |
| 22 | 21 | 77.8 | 27.5 | 35.4 |
| 23 | 18 | 79.8 | 26.0 | 37.6 |
| 24 | 19 | 83.5 | 27.6 | 33.0 |
| 25 | 19 | 82.4 | 25.4 | 30.9 |
| 26 | 21 | 81.8 | 27.2 | 33.2 |
| 27 | 20 | 80.1 | 28.2 | 35.2 |
| 28 | 22 | 80.0 | 29.8 | 37.2 |
| 29 | 23 | 83.5 | 29.4 | 35.3 |
| 30 | 22 | 83.9 | 29.8 | 35.3 |
| 31 | 21 | 80.5 | 27.8 | 34.7 |
| 32 | 21 | 82.3 | 29.8 | 36.2 |
| 33 | 20 | 80.8 | 29.9 | 34.9 |
| 34 | 22 | 79.0 | 28.8 | 36.4 |
| 35 | 30 | 79.5 | 28.2 | 35.5 |
| 36 | 18 | 81.9 | 27.8 | 33.9 |
| 37 | 17 | 81.9 | 27.8 | 34.1 |
| 38 | 25 | 79.0 | 25.4 | 32.3 |
| 39 | 28 | 77.3 | 28.4 | 36.8 |
| 40 | 22 | 83.1 | 30.9 | 37.2 |

TABLE 2—*Concluded*

| SUBJECT | AGE | CORPUSCULAR VOLUME | CORPUSCULAR HEMOGLOBIN | CORPUSCULAR HEMOGLOBIN CONCENTRATION |
|----------------|-----|-----------------------|---------------------------|--|
| | | <i>cu. microns</i> | <i>γγ</i> | <i>per cent</i> |
| 41 | 30 | 81.6 | 28.4 | 34.8 |
| 42 | 19 | 84.8 | 27.4 | 37.3 |
| 43 | 30 | 84.3 | 29.0 | 34.5 |
| 44 | 20 | 81.4 | 28.0 | 34.1 |
| 45 | 19 | 80.8 | 27.6 | 34.2 |
| 46 | 20 | 85.0 | 28.8 | 33.9 |
| 47 | 19 | 82.9 | 29.0 | 34.9 |
| 48 | 23 | 70.6 | 23.2 | 31.4 |
| 49 | 24 | 84.7 | 29.4 | 34.8 |
| 50 | 27 | 79.1 | 27.7 | 35.1 |
| Averages | | 80.1 | 28.0 | 35.2 |

Normal values for the volume and hemoglobin content of the average erythrocyte calculated from blood determinations in 100 healthy men residing in the southern United States were presented in an earlier report.¹⁴ In table 2 values derived from accurate blood determinations in fifty healthy young women, seventeen to thirty years of age residing in the South are recorded¹⁷. Figure 1 is a histogram showing the frequency distribution of corpuscular volumes in the fifty normal women. The mean ($80.1 \text{ cu.}\mu \pm 0.3$) and the median ($80.7 \text{ cu.}\mu \pm 0.3$) are practically equal and coincide with the peak of the curve. The standard deviation is $3.9 \text{ cu.}\mu \pm 0.2$ and the coefficient of variation 4.9 per cent. The minimal and maximal values were $70.6 \text{ cu.}\mu$ and $85.7 \text{ cu.}\mu$. These, however, are probably not a true index of the variation, extremes being usually the result of errors in technic or due to imperfection in the sample. Standard deviation is a much better measure of variation. Eighty-four per cent of the observed corpuscular volumes ranged between $76 \text{ cu.}\mu$ and $84 \text{ cu.}\mu$ which are the values one standard deviation on each side of the mean.

In table 3, corpuscular volumes in men and women are compared. It is interesting to find that the values in the two sexes are almost identical. Calculations based on blood determinations reported by other investigators show an almost equal similarity in the values for both sexes.

In Table 4 are found average, maximal and minimal values for corpuscular volume as calculated from the available reliable data reported by other investigators for normal young women seventeen to thirty years of age. It will be noted that the values calculated from blood determinations by Osgood and Haskins in Oregon, and Haden in Missouri are distinctly higher than those

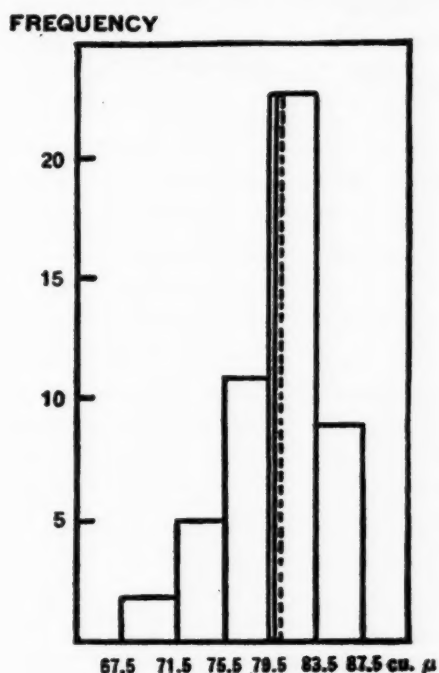


FIG. 1. HISTOGRAM SHOWING FREQUENCY DISTRIBUTION OF VARIATION IN CORPUSCULAR VOLUME IN 50 YOUNG WOMEN

The mean is indicated by a straight line, the median by an interrupted line.

found in Louisiana. A similar difference was noted in the values calculated for men.¹⁴ The cause of this difference is somewhat conjectural. The technic followed by Osgood and by me was practically the same and in each series solid potassium oxalate was used as the anticoagulant for the hematocrit determinations. Haden employed 1.6 per cent sodium oxalate solution.

Haden⁷ recently reported his investigations concerning the effect of various anticoagulants on cell volume. His experiments indicate that the employment of potassium oxalate, either in solid form or in a saturated solution, causes more shrinkage in cell volume than has been allowed for by either Osgood⁹ or by me^{13,17}. Osgood found that 20 mgm. of oxalate added to 10 cc. of blood caused a shrinkage of 3.5 per cent, while I allowed 3.7 per cent for

TABLE 3
CORPUSCULAR VOLUME VALUES FOR PERSONS IN LOUISIANA
(In cubic microns)

| | NUMBER | MEAN | MEDIAN | STANDARD DEVIATION, | COEFFICIENT OF VARIATION |
|----------|--------|------------|------------|------------------------|-----------------------------|
| | | | | | <i>per cent</i> |
| Men..... | 100 | 79.84±0.49 | 80.64±0.62 | 7.24±0.35 | 9.06 |
| Women... | 50 | 80.05±0.26 | 80.70±0.33 | 3.89±0.19 | 4.90 |

TABLE 4
VOLUME OF RED BLOOD CORPUSCLES IN 175 HEALTHY YOUNG WOMEN
(Expressed in cubic microns)

| AUTHOR | NUMBER OF SUBJECTS | AVERAGE | MAXIMUM | MINIMUM |
|---|--------------------------|---------|---------|---------|
| Osgood and Haskins (Oregon) ¹⁰ | 100 | 88.5 | 95.0 | 73.9 |
| Haden (Missouri) ⁶ | 9 | 92.8 | 98.1 | 90.5 |
| Gram and Norgård (Denmark) ⁶ | 6 | 88.0 | 91.3 | 85.1 |
| Bie and Möller (Denmark) ¹ | 10 | 81.7 | 85.8 | 77.9 |
| Wintrobe (Louisiana)..... | 50 | 80.1 | 85.7 | 70.6 |
| Averages..... | | 85.9 | 91.8 | 74.4 |

shrinkage when oxalate was added in this proportion, and 6.7 per cent when 40 mgm. of oxalate were added to 10 cc. of blood.

I have since repeated my experiments and in sixteen determinations found that potassium oxalate used in the proportion of 10 mgm. to 5 cc. of blood caused on the average, a shrinkage of 5.75 per cent in the volume of packed erythrocytes as compared with the volume found in heparinized blood. The amount of shrinkage was not the same for each specimen of blood, nor was

this variation in amount of shrinkage altogether correlated with the relative amount of blood plasma and the consequent variation in dilution.

The difference between the original correction for shrinkage and the correction which these subsequent experiments indicate should be made, is not of sufficient magnitude to account for the differences in the values for mean corpuscular volume in the three localities mentioned. Besides, there is an appreciable difference between the values derived from Osgood's blood determinations and those derived from my own investigations, although the amount of correction for shrinkage was in each case practically the same. It is just possible, then, that the differences in corpuscular volume are dependent on other factors than differences in technique⁴. It is hoped that further determinations will be made in other localities.

In summing up it may be said that, on the basis of the blood determinations presented in this and in an earlier paper¹⁴, the normal range of corpuscular volume in healthy young men and women residing in the Southern United States may be considered to be 75 cu. μ to 92 cu. μ , while the normal range for all localities is about 75 cu. μ to 95 cu. μ with an average of 85 cu. μ . Values above 95 cu. μ or below 75 cu. μ are probably a manifestation of abnormality.*

MEAN CORPUSCULAR HEMOGLOBIN

Mean corpuscular hemoglobin—the amount by weight of hemoglobin in the average red corpuscle—may be calculated by dividing the amount of hemoglobin, expressed in grams per 1000 cc. of blood, by the number of erythrocytes expressed as millions

* It is of interest to note that Ponder and Saslow have recently reported (*Jour. Physiol.*, **70**: 18-37. 1930) the average corpuscular volume, as determined by an accurate and laborious colorimetric method in ten individuals, to be 87.1 cu. μ . This corresponds closely to the mean value presented in this paper (85 cu. μ) and, contrary to the expressed opinion of Ponder and Saslow, suggests the adequacy of the hematocrit method for the determination of erythrocyte volume.

per cubic millimeter. The resulting value expresses mean corpuscular hemoglobin in micromicrograms.*

Calculations based on blood determinations in one hundred healthy young men residing in the South showed an average corpuscular hemoglobin of $29.2\gamma\gamma^{14}$. Average corpuscular hemoglobin based on 274 accurate blood determinations in different parts of the world was likewise $29.2\gamma\gamma$. Figure 2 shows the frequency distribution of corpuscular hemoglobin values based

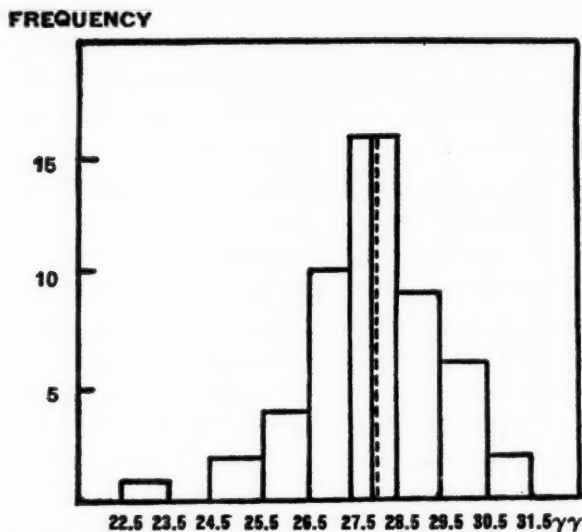


FIG. 2. HISTOGRAM SHOWING FREQUENCY DISTRIBUTION OF VARIATION IN CORPUSCULAR HEMOGLOBIN IN 50 YOUNG WOMEN

The mean is indicated by a straight line, the median by an interrupted line

on determinations in fifty healthy young women, seventeen to thirty years of age, residing in the Southern States. The mean ($27.96\gamma\gamma$) and the median ($28.00\gamma\gamma$), which are practically identical, coincide with the peak. The standard deviation is $1.55\gamma\gamma$ and the coefficient of variation is 5.54 per cent. Measuring by means of the standard deviation, the significant variation is 26.5 to $29.5\gamma\gamma$. Actually 86 per cent ranged between these values.

* A micromicrogram is the millionth of a millionth part of a gram, or grams $\times 10^{-12}$. It is abbreviated by the Greek letters $\gamma\gamma$.

In table 5 corpuscular hemoglobin values in the men and women examined in Louisiana are compared. These further determinations support the conclusions reached in an earlier report,¹⁴ namely, that there is no appreciable variation of corpuscular hemoglobin in respect to sex.

In table 6 corpuscular hemoglobin values calculated from data available for 175 healthy women seventeen to thirty years of age,

TABLE 5
CORPUSCULAR HEMOGLOBIN VALUES FOR MEN AND WOMEN IN LOUISIANA
(Expressed in micromicrograms)

| | NUMBER | MEAN | MEDIAN | STANDARD DEVIATION | COEFFICIENT OF VARIATION |
|---------------|--------|------------------|------------------|-----------------------|-----------------------------|
| | | | | | <i>per cent</i> |
| Men | 100 | 29.18 \pm 0.17 | 29.89 \pm 0.21 | 2.51 \pm 0.12 | 9.18 |
| Women . . . | 50 | 27.96 \pm 0.15 | 28.00 \pm 0.18 | 1.55 \pm 0.10 | 5.54 |

TABLE 6
CORPUSCULAR HEMOGLOBIN VALUES FOR HEALTHY YOUNG WOMEN
(Expressed in micromicrograms)

| AUTHOR | NUMBER OF WOMEN | AVERAGE | MAXIMUM | MINIMUM |
|--|-----------------------|---------|---------|---------|
| Osgood and Haskins ¹⁰ | 100 | 28.5 | 32.4 | 23.7 |
| Haden ⁶ | 9 | 31.3 | 31.8 | 30.0 |
| Gram and Norgaard ⁵ | 6 | 27.9 | 28.9 | 27.1 |
| Bie and Möller ¹ | 10 | 28.1 | | |
| Wintrobe | 50 | 28.0 | 30.9 | 25.4 |
| Averages | | 28.4 | 31.8 | 24.7 |

residing in different parts of the world, are given. The average of these corresponds closely with the average in 274 healthy men. There appears to be no great variation in corpuscular hemoglobin in the different localities considered. On the basis of the data at present available it can be said, then, that the normal corpuscular hemoglobin in the young adult is 28 or 29 $\gamma\gamma$, while the range of normal is 26.5 to 31.5 $\gamma\gamma$.

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION

The hemoglobin concentration of the average red corpuscle may be calculated by dividing the amount of hemoglobin, expressed in grams per 100 cc. of blood, by the volume of packed erythrocytes, expressed as cubic centimeters per 100 cc. of blood. The resulting value, multiplied by 100, expresses the mean corpuscular hemoglobin concentration in per cent.

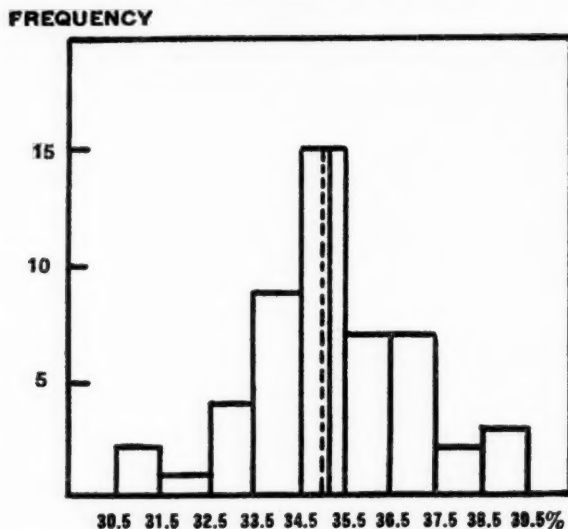


FIG. 3. HISTOGRAM SHOWING FREQUENCY DISTRIBUTION OF VARIATION IN CORPUSCULAR HEMOGLOBIN CONCENTRATION IN 50 YOUNG WOMEN

The mean is indicated by a straight line, the median by an interrupted line

In view of the fact that the manner of distribution of hemoglobin in the red cell is still unsettled, the term "concentration," implying as it does a state of solution, is used with some hesitation. However, since the relationship here considered is one of weight to volume, namely the amount of hemoglobin in proportion to the volume of the erythrocyte, the term "corpuscular hemoglobin concentration" or, more briefly, "corpuscular concentration" is used to express this relationship on the excuse of being descriptive and convenient and not with any implication as to the manner of distribution of the hemoglobin.

The average corpuscular concentration based on blood determinations in one hundred healthy young men in Louisiana, was 36.6 per cent, whereas the average of calculations based on 231 determinations on men of the same age group in different parts of the world was 34.6 per cent¹⁴. Figure 3 shows the frequency distribution of corpuscular concentration values based on blood determinations in fifty healthy women seventeen to thirty years of age, residing in the South. The mean (35.2 per cent) and the

TABLE 7

CORPUSCULAR HEMOGLOBIN CONCENTRATION VALUES FOR MEN AND WOMEN IN LOUISIANA

| | NUMBER OF SUBJECTS | MEAN | MEDIAN | STANDARD DEVIATION | COEFFICIENT OF VARIATION |
|----------|--------------------------|----------|----------|-----------------------|-----------------------------|
| | | | | | <i>per cent</i> |
| Men..... | 100 | 36.6±0.2 | 36.2±0.3 | 3.0±0.1 | 8.6 |
| Women... | 50 | 35.2±0.2 | 35.1±0.2 | 1.8±0.1 | 5.2 |

TABLE 8

CORPUSCULAR HEMOGLOBIN CONCENTRATION VALUES FOR NORMAL YOUNG WOMEN

| AUTHOR | NUMBER OF SUBJECTS | AVERAGE | MAXIMUM | MINIMUM |
|--|--------------------------|---------|---------|---------|
| Osgood and Haskins ¹⁰ | 100 | 32.2 | 33.7 | 29.0 |
| Haden ⁶ | 9 | 33.7 | 34.4 | 32.2 |
| Gram and Norgård ⁵ | 6 | 31.7 | 34.1 | 30.9 |
| Bie and Möller ¹ | 10 | 34.4 | | |
| Wintrobe..... | 50 | 35.2 | 39.0 | 31.4 |
| Averages..... | | 33.3 | 35.4 | 30.0 |

median (35.1 per cent) coincide with the peak. The significant variation on the basis of the standard deviation of 1.8 per cent, is 33.4 to 37.0 per cent. Actually 80 per cent ranged between these values.

In table 7 corpuscular concentration values in the men and women examined in Louisiana are compared. As was the case with corpuscular volume and corpuscular hemoglobin, corpuscular concentration shows no significant variation in respect to sex.

Corpuscular concentration values based on determinations made on 175 young women in different parts of the world are shown in table 8. The average of these values corresponds closely with the average in 231 healthy young men. From the data at present available it appears, then, that the average hemoglobin concentration of the red cell in the normal young adult is 35 per cent while the range of normal is approximately 33 to 39 per cent.

ILLUSTRATION OF METHOD OF CALCULATION

A sample of blood contains 5.5 million red blood cells per cubic millimeter, 15.5 grams of hemoglobin per 100 cc. of blood, and 45.2 cc. of packed red cells per 100 cc. of blood. Then,

$$\text{Mean corpuscular volume is } \frac{452}{5.5} = 82.2 \text{ cu. } \mu$$

$$\text{Mean corpuscular hemoglobin is } \frac{155}{5.5} = 28.2 \text{ } \gamma \gamma$$

$$\text{Mean corpuscular hemoglobin concentration is } \frac{15.5}{45.2} \times 100 = 34.3 \text{ per cent}$$

It is not within the scope of this paper to present the details of corpuscular volume, corpuscular hemoglobin and corpuscular concentration determinations in the various types of anemia. It may be mentioned, however, that I have found these calculations of great value in affording a clearer conception of the alterations in the physical state of the erythrocytes associated with various diseases. In the diagnosis of pernicious anemia and sprue corpuscular volume determinations have proved to be of particular value. Although mean cell diameter is not always significantly greater than normal in these diseases¹², I have found mean corpuscular volume always distinctly greater than normal during the stages of relapse. Differences in the size of erythrocytes are probably present in all dimensions. Thus differences which may be insignificant when only one dimension such as diameter is measured, are readily noted when corpuscular volume is calculated. Although it might be expected that the small and distorted cells found in the blood of patients suffering from perni-

cious anemia would tend to lower the mean volume of the cells, actually the effects of even marked anisocytosis and poikilocytosis have not been found to be sufficient to reduce mean corpuscular volumes below values which are significantly higher than normal.

Finally it may be mentioned that a most interesting feature of blood determinations in a large series of anemias of all types is the possibility that all anemias may be subdivided into four distinct classes on the basis of differences in the size and hemoglobin content of the erythrocytes.¹⁹

SUMMARY

The inaccuracy and inadequacy of hematologic technic is pointed out and the vagueness and inaccuracy of the color and other indices discussed. For reasons of simplicity, increased clarity and greater accuracy, the direct calculation of the average volume of the red blood corpuscle, the amount of hemoglobin it contains, and the "concentration" of hemoglobin in the cell is proposed. Simple methods for such calculations are presented.

Normal values for corpuscular hemoglobin, corpuscular volume and corpuscular hemoglobin concentration, calculated from accurate blood determinations carried out in different parts of the world, are given. These may be summarized as follows:

1. The quantity of hemoglobin in the average erythrocyte (mean corpuscular hemoglobin) of the blood of a healthy young adult is 28 to 29 micromicrograms. The range of normal is 26.5 to 31.5 $\gamma\gamma$.

2. The volume of the average erythrocyte (mean corpuscular volume) of the blood of a healthy young adult residing in the southern United States is 75 cu. μ to 92 cu. μ . In other localities for which blood determinations are available, the average corpuscular volume appears to be somewhat greater than this. On the basis of accurate data at present available the extreme variation of corpuscular volume in all localities may be considered as being 75 to 95 cu. μ , while the average corpuscular volume may be taken to be 85 cu. μ .

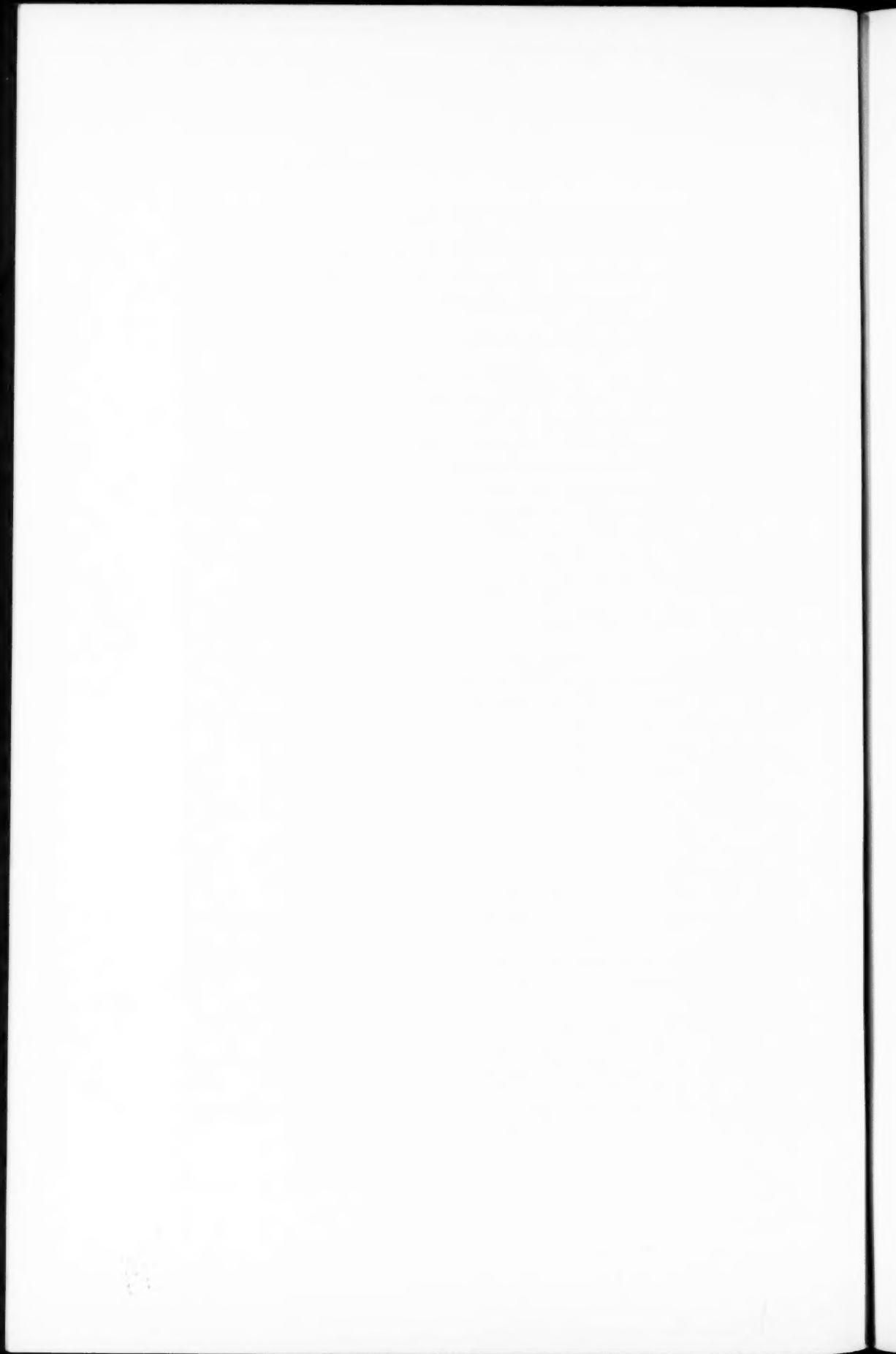
3. The proportion of hemoglobin in the average erythrocyte (mean corpuscular hemoglobin concentration) of the blood of a healthy young adult is 33 to 39 per cent and averages 35 per cent.

No differences in corpuscular volume, corpuscular hemoglobin, or corpuscular hemoglobin concentration have been observed in respect to sex.

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GASTRIC MANIFESTATIONS OF LYMPHATIC ALEUKEMIA (PSEUDOLEUKEMIA GASTRO- INTESTINALIS)*

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Development of major clinical symptoms in association with extensive infiltration of the stomach in various forms of lymphomatous disease (lymphomatosis) is considered uncommon. A presumptive diagnosis may be ventured by biopsy of the local mass or of the peripheral node at operation. Without such biopsy and in the absence of a palpable spleen or peripheral masses or of a leukemic blood picture, clinical diagnosis may become well nigh impossible. Gastric deformities reproduced on the roentgenograms may easily be interpreted as due to cancer.

REPORT OF CASE

A male, seventy-one years of age, a mild diabetic for about twenty years, obese and comparatively healthy, complained of a continuous dull pain in stomach, in November, 1921. This had been present for many years and was relieved only partially by soda. In January, 1923, the condition became acutely aggravated. Self-induced vomiting relieved the distress. The vomitus contained particles of food ingested two days before but gastric lavage sometimes failed to recover raisins eaten the night before. Gastric analysis showed free hydrochloric acid 38° and total acidity 54°. Stools were negative for occult blood. There was indefinite soreness over the epigastrium but a mass could not be palpated. Roentgen examination showed a slightly enlarged, hypotonic stomach with a retention of barium for twenty-eight hours. The pylorus could not be made to fill properly and the duodenal cap was poorly made out. A beginning carcinoma or a concealed ulcer, at the pylorus, with retention was considered a possibility, both clinically and roentgenologically. No definite di-

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agnosis was reached at the time, however, and the patient continued to be treated symptomatically with indifferent results. More or less gastric distress was present every day, especially after meals. December 1, 1923, the patient developed acute epigastric pain with vomiting and unusual amounts of gas. Roentgen examination at this time showed an enlarged stomach with sluggish peristalsis and spastic deformity at the pylorus. The duodenal cap showed a definite filling defect. A small amount of barium residue was noted at the end of forty-eight hours. Roentgen diagnosis of chronic duodenal ulcer with acute retention was made. Laparotomy was performed. A large mass about the size of an orange obstructing the orifice was found in the region of the pylorus. The regional lymph nodes were enormously enlarged, quite hard on palpation and extensively involved. The head of the pancreas was also involved in the mass. The liver showed no evidence of metastasis. A gastro-enterostomy was performed. Diagnosis of probable carcinoma of the stomach was made without biopsy. Before operation, the hemoglobin was 92 per cent, erythrocyte count, 5,400,000 and leukocyte count, 18,300. The latter was 7,250 two weeks later. Three weeks later, a second laparotomy was performed to relieve intestinal obstruction due to adhesions. The gastric tumor was noted to be considerably reduced in size at this time.

Subsequent history was like that of an usual mild diabetic whose urine sugar was controlled by diet and whose blood sugar was never over 0.285 per cent. The patient was well built, rather obese, weighing about 78 kilograms. He presented himself, from time to time, for various minor ailments such as chronic urethritis, bilateral otitis media, impairment of vision due to hyaline degenerative spots in the left macula, et cetera.

In December, 1928, the patient for the first time complained of intermittent heart beats. Electrocardiograms showed left and right ventricular extrasystoles. Blood pressure was 110 mm. systolic and 55 mm. diastolic and pulse 58 per minute. The cardiac condition became progressively worse and in April, 1929, edema of both legs and irregularity of pulse developed. In August, 1929, the enlargement of axillary and inguinal lymph nodes was noticed for the first time. In September, 1929, hemoglobin was 70 per cent, erythrocyte count, 4,040,000 and leukocyte count, 15,600 with a differential count showing neutrophils, 49 per cent, lymphocytes, 47 per cent, monocytes, 3 per cent and eosinophiles, 1 per cent.

On January 31, 1930, swelling of the right abdomen and swelling and pain in the left leg developed. Regional glands increased in size and number, some in the axilla having reached the size of a hen's egg. A few nodes, discrete, movable and fairly soft in consistence, were palpable in the neck. The hemoglobin was 45 per cent, erythrocyte count, 3,080,000, leukocyte count, 18,500 with a differential count showing 54 per cent neutrophils, 43 per cent lymphocytes and 3 per cent monocytes. No pathological forms of lymphocytes were noted in the study of the blood smears. Biopsy of an axillary node was done on February 4

to determine whether the cause of the adenopathy was due to Hodgkin's disease, leukemic infiltration or metastatic carcinoma. Histologic diagnosis was leukemic infiltration of the lymph node.

On February 10, 1930, the patient died of circulatory failure.

NECROPSY

The body was that of a well developed, obese, adult, white male 170 cm. long and approximately 82 kgm. in weight. The body was embalmed and several trocar punctures were present in the abdominal wall. The skin and the mucous membrane showed a moderate degree of palor. A long surgical scar 15 cm. in length was noted in the upper abdominal wall to the right of the mid-line. The cervical nodes were palpable, those on the left anterior triangle were quite prominent. The axillary nodes were swollen and protruded prominently on both sides along the anterior axillary line, some approximating 6 cm. in diameter. The inguinal nodes were likewise enlarged. They were discrete, well encapsulated and soft. The cut surface was pinkish-white, homogeneous and rubbery in consistence. The subcutaneous fat was 2 cm. in thickness. The subcutaneous tissue was swollen and edematous. The scrotum and the penis were greatly edematous. No jaundice, cyanosis or hypostasis was noted.

Incision was limited to the upper abdomen.

The peritoneal cavity contained a large amount of fluid mixed with embalming liquid. The appendix was 10 cm. long and 1 cm. in average diameter. It was firm, solid (figs. 3a, b) and easily broken. The cut surface consisted of a solid, homogeneous, white, cellular tissue surrounded by a thin muscular layer. The liver border extended 2 cm. from the costal margin.

Each pleural cavity contained a large amount of clear, amber fluid, estimated at 1500 cc. Both lungs were collapsed against the spinal column.

The heart and the lungs were not removed for examination. Manual examination revealed a slightly enlarged heart with a definite hypertrophy of the left ventricle which was firmly contracted.

The lungs were both collapsed and shrunken. They showed no areas of consolidation.

The spleen weighed approximately 120 grams. The capsule was grayish and slightly thickened. The cut surface showed a dark red, soft pulp. The corpuscles were numerous and quite prominent, ranging in size from 1 to 3 mm. in diameter and especially numerous beneath the capsule.

The liver appeared about normal in size. It was pale, grayish and soft. The cut surface was swollen, cloudy and showed a pale yellowish-brown parenchyma, regularly studded with hemorrhagic spots which surrounded the lobular centers.

The stomach was definitely enlarged weighing approximately 750 grams and measuring 46 cm. from the cardia to the pyloric ring and 25 cm. in greatest circumference. The serosa was thickened and fibrous. Many enlarged lymph

nodes were adherent along both the lesser and the greater curvatures. The entire wall was tough and leathery in consistence (fig. 1). The mucosa was enormously hypertrophied (fig. 2) and on section, white and diffusely cellular and up to 1.5 cm. in thickness. The rugae along the cardiac half of the stomach were in deep and thick folds, like the convolutions of the brain, up to 2 cm. in height and 2.5 cm. in thickness. In the distal half, the mucosa was velvety, showed a few wide, longitudinal furrows along the lesser curvature and a few, diffuse, ill-defined elevations. Proximal to the pyloric orifice was a round, tumor-like mass, 6 by 6 by 5 cm. which was found lying within the wall but

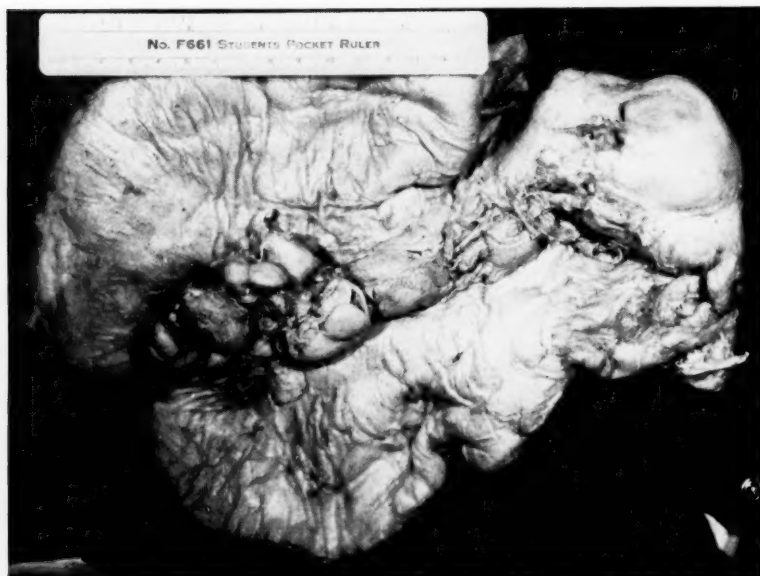


FIG. 1. Stomach, peritoneal surface: Thickened, fibrous, leathery serosa; several enlarged lymph nodes and a large lymphoma at the pylorus.

protruding into the pyloric antrum and infiltrating into the ring. The cut surface of this mass showed a soft, pinkish-gray, homogeneous lymphoid tissue. Along the dependent parts of the greater curvature was a functioning gastro-enterostomy stoma.

The mucosa of the small intestine was edematous. The lymphoid follicles were conspicuous throughout. The Peyer's patches showed irregular hypertrophy and elevations up to 1 cm. in height. The colon was somewhat dilated. The wall was thinned. The mucosa was studded with many nodular lymphoid follicles up to 5 mm. in diameter and 3 mm. in height. At the cecum, there were two rounded masses, one, 4 cm. in diameter and 3 cm. in height and the other,

a rounded, umbilicated elevation 3 cm. in diameter and 2 cm. in height, around the orifice of the ileocecal valve. The mucosa was reddened and hemorrhagic while the surface of the mass was roughened, hemorrhagic and showed superficial gangrenous discoloration (fig. 4).

The head of the pancreas was replaced by a soft, white tumor-like tissue. The splenic vein was filled with a firm, organized coagulum. Small irregular areas of white, soft tumor-like tissue were scattered in the substance of the pancreas.

The adrenal glands were normal.

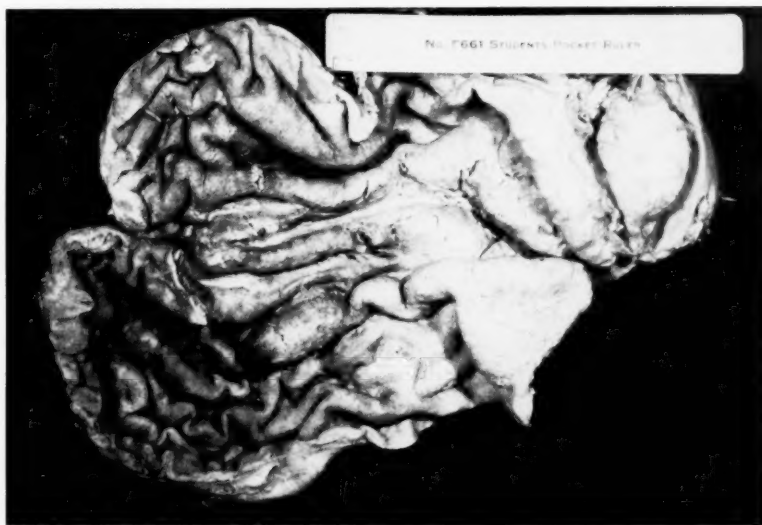


FIG. 2. Stomach, opened: Rugal folds resembling "the convolutions of the brain;" note thickness of the mucosa; note a mass at the pyloric end with narrowing of the lumen.

The right kidney weighed 160 grams. The capsule was grayish, about 2 mm. in thickness and adherent. It stripped with some difficulty and left a whitish, roughened, mottled surface. The cut surface revealed a grayish parenchyma peppered with punctate hemorrhagic areas. The surface was swollen and markings were rather indistinct. The left kidney with the capsule was 15 by 8.5 by 8.5 cm. and weighed 700 grams. The capsule was fibrous, leathery, 4 mm. in thickness and apparently infiltrated by tumor cells. It was firmly adherent to the cortical surface which was grayish, roughened and covered with residual adhesions. The cut surface was waxy and swollen. The cortex was 1.5 cm. in width and mottled with many punctate hemorrhages and markedly

infiltrated with grayish tumor-like tissue. The pyramids were dark red and stood out conspicuously against a background of a whitish, waxy parenchyma. The mucosa of the pelvis was swollen and hemorrhagic.

The mesenteric, retroperitoneal and regional lymph nodes along the stomach, pancreas, hilum of the liver as well as the cervical, axillary and inguinal nodes were enormously increased in number and showed enlargement varying in diameter from 1 cm. to 6 cm. They were discrete but found in conglomerate masses. They were pinkish-gray in color, uniformly soft, friable and appeared quite cellular. Involvement of the posterior gastric and peripancreatic nodes was most extensive.

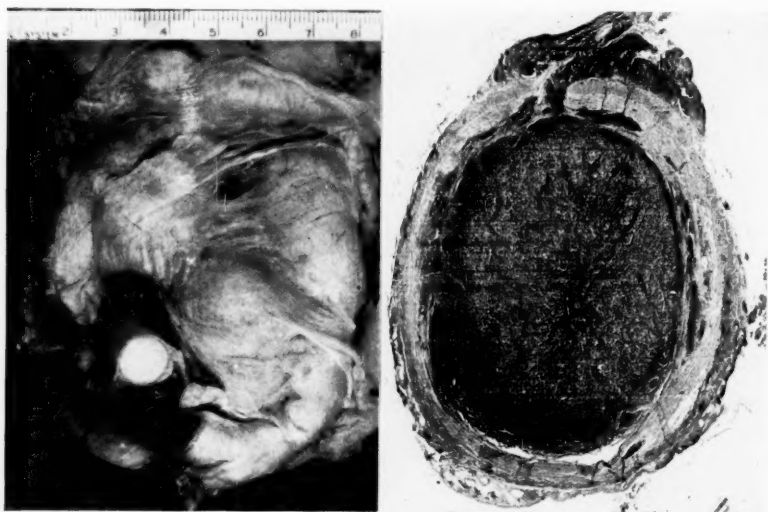


FIG. 3a. Cecum, peritoneal aspect: Appendix buried in adhesions; note a cross section of the appendix; several enlarged nodes.

FIG. 3b. Appendix showing a massive leukemic growth obliterating the lumen and replacing the entire mucosa and submucosa; infiltrations into the muscularis and out into the serosa and meso-appendix.

Diagnosis (limited to the abdominal viscera)

1. Leukemic infiltration and lymphoma of the stomach and intestines.
2. Leukemic lymph nodes (mesenteric, retroperitoneal, perigastric, pancreatic, cervical, axillary and inguinal).
3. Leukemic infiltration of the kidneys and the pancreas.
4. Chronic passive congestion and cloudy swelling of the liver.
5. Gastro-enterostomy.
6. Thrombosis of the splenic vein.
7. Bilateral hydrothoraces and ascites.

Microscopic study

Stomach: The mucosa of the stomach showed areas of superficial necrosis over the periphery. Beneath, the glandular architecture was completely obliterated by diffuse invasion of lymphocytes which freely infiltrated into the submucosa (fig. 5) and aggregated into many pseudofollicular masses (fig. 6) up to 1 mm. in diameter. The muscularis was diffusely hypertrophied. The lymphocytes infiltrated deeply into the muscle wall and out into the thickened

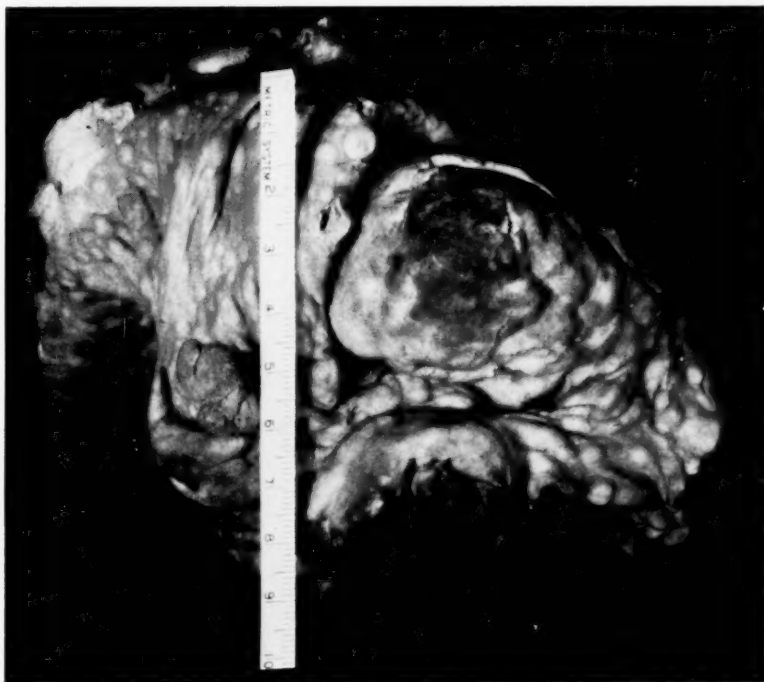


FIG. 4. Cecum, opened: Two large lymphomatous masses; numerous small lymphoid nodules.

serosa, forming multiple nests of cells in scattered areas. The cells were uniform in size and shape, of the undoubted adult lymphocyte type, showing a large round nucleus with coarse hyperchromatic strands of chromatin and showed no mitosis (fig. 7). This, essentially, represented a sectional view of an average ruga.

The tumor-like mass at the pylorus showed a histologic structure identical with that seen in the lymph nodes. It was a diffuse mass of the lymphocytes supported by a scanty stroma carrying a few fine capillaries. A few large, pale

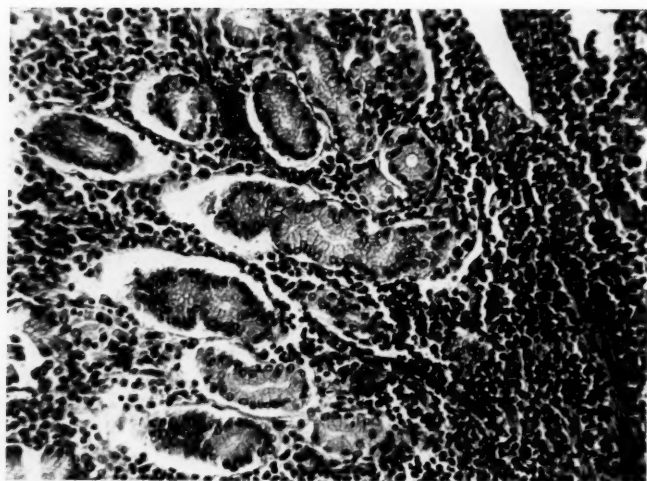


FIG. 5. High power picture of the gastric mucosa

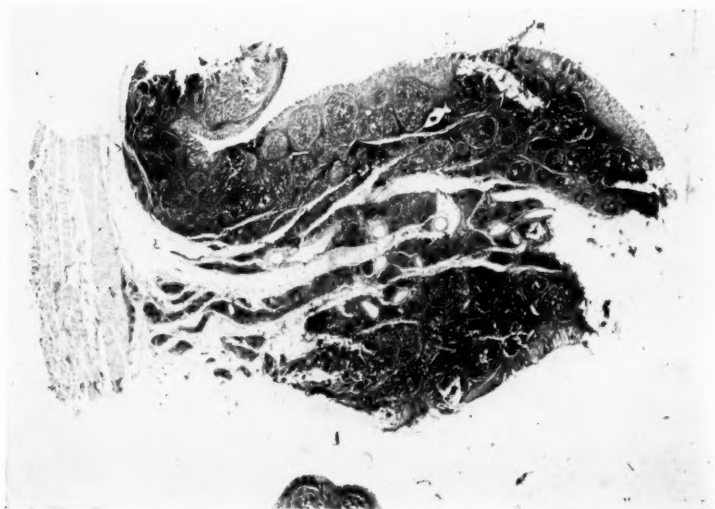


FIG. 6. Low power section of a mucosal fold of the stomach; note many pseudo-follicles immediately beneath the glands and diffuse hyperplasia of lymphocytes and invasion into the muscularis.

staining cells of the reticulo-endothelial type might be seen through the mass of the more dark staining lymphocytes. No mitotic figures were observed. Neither were there any multi-nucleated giant cells of the Dorothy Reed type.

Lymphoid hyperplasias and infiltrations of the identical character were noted in the terminal ileum and the cecum.

The pancreas showed diffuse interlobular masses of lymphocytes which infiltrated also between acini. Interstitial fibrosis and hyaline degeneration of the islands of Langerhans were also noted.

The kidney showed the most striking picture of leukemic infiltration through-

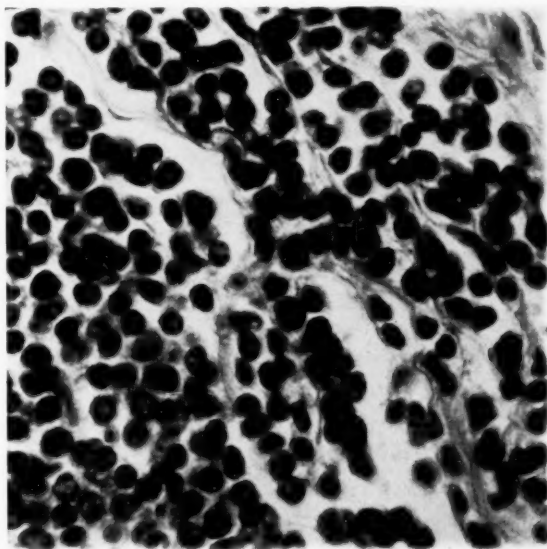


FIG. 7. A higher power view of the lymphocytes; note the uniformity of size, shape, staining quality, et cetera.

out the cortex and deep in the medulla, the lymphocytes spreading in large masses and cords.

The liver and the spleen showed the minimal perivascular accumulation of lymphoid cells. Diffuse hemorrhage at the lobular centers with atrophy of the hepatic cords.

Microscopic diagnosis

1. Leukemic infiltration of the stomach, intestines, spleen, liver, kidneys, pancreas and lymphatic nodes.
2. Chronic passive congestion of the liver.
3. Hyaline degeneration of the islands of Langerhans of the pancreas.

COMMENT ON THE CASE

In the light of the necropsy, the following observations may reasonably be drawn:

The gastric symptoms must be attributed to the leukemic changes in the wall of the stomach which consisted essentially of the diffuse leukemic infiltration of the mucosa and the localized lymphomatous growth at the pylorus. It is reasonable to conclude that these symptoms had been present, at least, since early in 1921 or nine years prior to death and that the roentgen findings in the stomach in 1923 were due to these leukemic changes. The pyloric tumor and the enlarged regional lymph nodes found at operation were leukemic masses and not cancerous growth and regional metastases, as clinically diagnosed. In the absence of a leukemic blood picture and of demonstrable peripheral adenopathy, a correct diagnosis was clinically impossible at the time. Without biopsy at the time of laparotomy, a diagnosis of probable carcinoma of the stomach seemed reasonable. Indeed, the clinical and roentgen observations as well as the operative findings were strikingly suggestive of gastric cancer. A five-year cure might have been thus recorded, illustrating again the vital importance of biopsy, if at all possible, in even the most obvious cases of clinical cancer.

Noteworthy also is the fact that advanced as was the involvement of the lymph nodes in and about the wall of the stomach and the pancreas at the time of laparotomy in 1923, peripheral adenopathy was not noted until five years later, a clinical observation which, while not uncommon in leukemias and aleukemias, nevertheless, deprived a possible means of early diagnosis in this case. When the peripheral nodes finally became demonstrable and a biopsy diagnosis of lymphatic aleukemia was made, it was considered rather as an independent clinical finding than either as an etiologic factor of the existing cardiac lesion or as the cause of the gastric symptoms which had long subsided following the gastro-enterostomy seven years before.

GENERAL DISCUSSION

The involvement of the stomach in various forms of the so-called lymphomatosis is rare in routine experience although it is

not infrequently reported in literature. When encountered at necropsy, unless some guiding clinical data are at hand, the exact anatomical diagnosis may be difficult, while the histological picture may be equally confusing. Thus, Hodgkin's granuloma, lymphosarcoma, leukosarcoma, leukemia and pseudoleukemia, all may similarly involve the stomach. With minor variations, they may present an identical general gross appearance. This may be represented by a localized thickening or induration of the wall or one or more circumscribed tumors and nodules, with or without ulceration. Sometimes this condition is characterized by a diffuse thickening of the wall with prominent, deep, sinuous folds of the thickened mucosa or rugae, usually described as resembling "the convolutions of the brain," with or without localized nodular elevations or by one or more circumscribed flat thickenings of the mucosa. The regional lymph nodes are invariably involved. Microscopically, the general granulomatous picture with areas of fibrosis in Hodgkin's disease, the invasive character in lymphosarcoma, the malignant behavior with a leukemic blood picture in the so-called leukosarcoma and the characteristic organ infiltration and blood picture in leukemia, may each determine a final diagnosis. The fact is, however, that a differential pathological diagnosis between these conditions, presenting as they do a similar anatomical and clinical picture and often a confusing microscopic behavior, is by no means simple but difficult at times.

Because of this similarity and of the apparently close etiological relationship which exists between these conditions, a certain school of pathologists prefer to group them all under a general term of lymphoblastoma¹² while others consider them as lymphosarcomas or malignant lymphomas. The final diagnosis is thus made a matter of individual training and conception, particularly when it is done solely on the anatomic and histologic basis without the clinical data including the blood picture. On the other hand, Simonds²⁰, concluding his recent review on Hodgkin's Disease, makes the following timely statement:

"The progress thus far made in the study of diseases of the lymph glands has been the result of the splitting up of the composite group of pathologic conditions originally described by Hodgkin, into leukemia, pseudoleukemia,

lymphosarcoma and Hodgkin's disease. There is a tendency to recombine these diseases into a common group. Until their etiology is discovered, the final solution of the problem of their relation to each other will be furthered by rigidly maintaining the histo-pathologic criteria already devised for their differentiation."

HODGKIN'S GRANULOMA AND LYMPHOSARCOMA OF THE STOMACH

Many cases of Hodgkin's granuloma involving only the stomach or the gastro-intestinal tract have been reported during the last two decades.

Schlagenhauer,¹⁹ Eberstadt,⁶ Reimann¹⁸ and Partich¹⁶ made important contributions on this subject. More recently, Hayden and Appelbach¹¹ reviewed twenty-six such cases with cardinal anatomic lesions practically entirely limited to the digestive tract. Microscopically, the representative lesion in these cases is typically granulomatous and as such, differentiation from the remaining groups of lymphomatous growths appears less difficult.

Of 592 sarcomas of the digestive tract collected by Goldstein⁹ up to 1921, 265 occurred in the stomach. According to various authorities, sarcoma of the round cell type constitutes by far the most predominant among the sarcomas of this organ and the majority of the so-called round cell sarcomas, most probably, belong to a group identified as lymphosarcoma.¹³ Indeed, in this group, are probably included an appreciable number of leukemic growths, pseudoleukemias as well as Hodgkin's granuloma of the more cellular type.²⁴

LEUKEMIC INFILTRATION OF THE STOMACH

A characteristic attribute of leukemia is its tendency to infiltrate the visceral organs, notably the spleen, the liver, and the kidneys, as well as the lymphoid tissue everywhere, to a greater or less extent. The degree of infiltration is a variable factor; one or more of these organs may show a greater or less involvement than others. The blood itself may not be an exception, a normal count or a normal morphology of the leukocytic elements or both, persisting throughout life. Localized lymphoid hyperplasias in the wall of the gastro-intestinal tract in leukemia, if carefully searched for at necropsy, are probably not uncommon, although

clinical symptoms arising from such are seldom encountered. Diffuse or massive leukemic infiltration of the gastric wall with the production of major symptoms there from is undoubtedly infrequent. Leudet¹⁴ described very curious lesions (infiltration) consisting of mucosal elevations and soft, round masses in the wall of the stomach at autopsy in a case of leukemia. DeRoth⁵ stated that when the stomach was affected in leukemia, there was a considerable thickening of the mucosa which became wrinkled and resembled the "convolutions of the brain." Such a degree of involvement of the gastric wall, however, is quite uncommon in proven cases of leukemia.

Symmers²⁴ made this observation:

"In true lymphatic leukemia the lymphoid structures of the gastro-intestinal tract rarely, if indeed they ever, undergo a degree of hyperplasia, even remotely comparable to that just described (pseudoleukemia gastrointestinalis). In nineteen cases of chronic lymphatic leukemia of which I have records, the gastro-intestinal lymphoid structures were practically unchanged."

The archives in the Department of Pathology of the University of Minnesota reveal that, of 12,396 necropsies performed from 1900 to 1928, inclusive, there were seventy-seven cases of leukemia recorded, of which fifty-one cases were of the lymphatic type and twenty-six of the myelogenous type. Of the fifty-one cases of lymphatic leukemia, two showed a stomach with a local nodular elevation or thickening and two ulcers. Of the twenty-six of myelogenous leukemia, only one showed a local thickening. The following clinical case²⁶ is cited as a pertinent example.

The patient, an elderly white man of sixty-two years was admitted to the University Hospital in June, 1929, complaining of gastric distress, with loss of weight, abdominal distension with visible peristaltic waves and absence of free gastric hydrochloric acid, presenting a clinical picture of gastric cancer. His leukocyte count ranged from 13,000 to 40,000 per cubic millimeter with 90 per cent of lymphocytes, on the basis of which a diagnosis of chronic lymphatic leukemia was made. Roentgen study of the gastro-intestinal tract revealed an enormous thickening of the rugal folds throughout the entire mucosa of the stomach and first portion of the duodenum. A roentgen diagnosis of probable chronic hypertrophic gastritis or multiple polyposis with malignant degeneration was made, and an exploratory laparotomy was performed. The stomach was enormously dilated. The wall was diffusely thickened and nodular. There

were many enlarged lymph nodes along the greater curvature. Thickening extended all along the duodenum and the small intestine to the cecum where large masses were palpated within the lumen. Biopsy of a regional lymph node showed leukemic hyperplasia.

Sternberg²¹ recognized an intermediary form between the true lymphosarcomas and the leukemias and designated it as "leuko-sarcoma." Flashman and Leopold⁸ reported a case of this kind in which a primary lymphoma was found in the pelvis without a leukemic blood picture. Following intensive roentgenotherapy with disappearance of the mass and temporary clinical improvement, the patient returned with a rapidly progressive leukemic blood. At autopsy, the stomach showed an enormous thickening of the mucosa with a typical configuration likened to the brain convolutions. Warthin²⁷ advocating the theory of the neoplastic nature of leukemia, reported a case in a man of thirty years, suffering from jaundice, constipation and gastric symptoms, in whom "between 80 and 90 per cent of the 90,000 white cells were atypical mononuclears" and who died after operation. At autopsy, the stomach as well as the small intestine showed a diffuse growth, in general resembling a lymphosarcoma. The growth was fairly vascular and the cells showed a marked tendency to invade the vessels. In the stomach, the tumor cells could be seen infiltrating the muscle. Warthin advanced a name "leukoblastoma" for this type of lymphatic manifestation.

"PSEUDOLEUKEMIA GASTRO-INTESTINALIS"

Conheim⁴ recognised anatomic characteristics in leukemia without changes in the blood and suggested the name pseudoleukemia. Cabot¹⁵ defined it "as a hyperplasia of specially hemopoietic tissue closely akin to leukemia, in fact, distinguished therefrom solely by the absence of leukemic changes in the peripheral blood." Sternberg²² emphasized distinct features of pseudoleukemia and separated it as an entity from Hodgkin's disease. Ewing⁷ considers "true pseudoleukemia" as a systemic aleukemic lymphomatosis while Warthin¹⁵ designates the condition as a generalized (or localized) aleukemic lymphocytoma. Of the gastro-intestinal form of pseudoleukemia, Ewing makes the following observation:

"The gastro-intestinal tract is a seat of a remarkable form of primary lymphoid hyperplasia which lacks the destructive character of lymphosarcoma and fails to give lymphocytosis in the blood. The process may be chiefly limited to a portion or involve the whole of the gastro-intestinal tract or it may be associated with wide spread lesions of the most other lymphoid structures."

Wells and Maver²⁸ collected seven cases from the literature added one of their own and reported them under the title of pseudoleukemia gastro-intestinalis and thus, for the first time, brought to the attention of the pathologist and the clinician an interesting group of cases which they considered as "a division of the general group of cases that present the anatomical and symptom complex of Hodgkin's disease." Hayden and Appelbach,¹¹ however, considered these and other cases subsequently published under the same title, as belonging to an entity, entirely distinct from gastro-intestinal lymphogranulomatosis or Hodgkins disease.

Analyzed in the light of a more recent conception, at least a majority of these cases apparently represent a gastro-intestinal manifestation of lymphatic aleukemia and should be so designated rather than grouped under that ambiguous and misleading term, pseudoleukemia, which embraces also the other group represented by a minority of cases showing a sarcomatous transition or granulomatous nature. This assumption seems to be warranted from the histologic and hematologic data, meager as they are in most of the instances, afforded in the original reports cited by Wells and Maver and in those, subsequently, published by others. Thus, Carrington³ reporting a case of Hodgkin's disease, stated "microscopically, sections of the stomach showed the structure of lymphoma, the growth consisting of abundant cells resembling leukocytes with, however, little or no evidence of reticulated stroma."

Hadden¹⁰ described the growth as "strictly limited to the glands of the stomach without ulceration. On microscopic examination, the glands of the small intestine showed abundant small round cells, evidently a hyperplasia of the normal lymphatic tissue. There was no sign of invasion to neighboring parts." Pitt's¹⁷ case showed microscopically, "the growths in the stomach

and elsewhere were found to consist of a dense collection of small cells in an adenoid reticulum which was with difficulty discoverable." The structure of the lesions in Symmer's²⁵ case was "peculiar, consisting of extensive multiplication of lymphoid follicles which became greatly enlarged and eventually fused—a true lymphadenoma, yet the cells in the centers of these aggregations of follicles were medium sized lymphocytes. Mitoses were missing. The process showed no capacity to invade resisting structures."

Stoerk²³ reported a case in which "by exclusion we come to a diagnosis of pseudoleukemia" and in which a microscopic study showed "the round cells forming the infiltration correspond in form and size and especially in size of the nucleus and protoplasmic content, to the type of lymphatic cells. In some places, there were found somewhat lighter cells which could not be differentiated from the cells of the adenoid tissue." On the other hand, he believed that there were some border line cases where he felt justified in making the following observation: "while in most of the cases, the cell types clearly resemble lymphatic cells, there are cases where the cells, because of their irregular forms, size of nuclei, non-uniform staining, resemble more sarcomatous than adenoid types. The problem is not easy to solve and we are probably justified to assume that we have to deal here with an intermediate stage between pseudoleukemia and lymphosarcoma, etc." In this class the case reported by Briggs and Elliott² might be more properly included while the case of Wells and Maver was an undoubted instance of Hodgkin's disease with chief manifestations in the gastro-intestinal tract.

From the foregoing discussion, it would seem reasonable to conclude that the case here reported is that of lymphatic aleukemia presenting major clinical symptoms referable to the stomach due to diffuse leukemic infiltration of the gastric wall without lymphemia or the involvement, at first, of the peripheral nodes. It exemplifies Ewings' true pseudoleukemia or systemic aleukemic lymphomatosis and Warthin's¹⁵ generalized aleukemic lymphocytoma with its primary clinical and pathological manifestations in the stomach. Contrary to Warthin's experience that "lym-

phocytoma of the stomach or intestine and mesenteric glands has been one of the most common forms," search of references in the literature, supported by my experience and that of others, has shown that the condition comparable to my case, in all its essential features, seems strikingly rare.

Clinically, the majority of the patients suffering from this type of gastric lesion complain of vague symptoms referable to the stomach. Pyloric obstruction may be the chief presenting symptom. Free hydrochloric acid is absent in many of the cases reported. Correct diagnosis is probably impossible. With positive laboratory or roentgen findings, a presumptive diagnosis may be possible. Baldridge and Awe¹ observe that "when there is an actual invasion of the stomach wall by one of the non-circulating type [of lymphoma] the correct diagnosis may be established only by the discovery of an involvement of the peripheral lymph nodes . . . without involvement of the peripheral lymph nodes the differentiation is apt to be impossible, clinically." Differential diagnosis from cancer, if at all possible, is of distinct therapeutic and prognostic importance. Differentiation between the various sub-groups of lymphoblastomata, if obtainable, may be of prognostic value. The roentgen rays offer the most direct method of visualizing the gastric lesion. Deformities and filling defects, thus visualized, are invariably interpreted as cancer. The case reported by Briggs and Elliott² may be cited as a typical example, presenting a characteristic roentgen picture of diffuse carcinoma of the stomach. Holmes, Dresser and Camp¹² reported a series of eight cases of "lymphoblastoma" involving the stomach. Of the six cases showing a definite filling defect in the wall of the stomach, five were interpreted as carcinoma and one as lymphoblastoma, the latter only in the light of a previous biopsy diagnosis on a peripheral lymph node. Demonstration of deep, heavy rugal impressions, by a proper technic, such as shown in our case of clinical lymphatic leukemia, cited above,²⁶ together with such pertinent clinical and laboratory data as a positive blood picture, biopsy diagnosis, etcetera, may be of diagnostic value. There is, however, no pathognomonic roentgen picture of the stomach in the gastric manifestation of this disease.

CONCLUSIONS

A case of lymphatic aleukemia showing an early involvement of the stomach with obstructive symptoms is reported. The chief clinical interest lies in the fact that the condition resembled gastric cancer so closely that without necropsy, a correct diagnosis could not have been made.

Leukemic infiltration of the gastric wall with or without a positive blood picture, is a definite, though uncommon, clinical and pathological entity and should be differentiated from other forms of allied lymphomatous condition.

The term "pseudoleukemia gastro-intestinalis" represents, no longer, a distinct histopathological entity but merely a group of heterogeneous lymphomatous lesions of the digestive tract and should be discarded.

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EDITORIAL

DIPHYLLOBOTHRIUM LATUM

The development and spread of civilization, knowledge, and commerce play decided parts in the infestation of both man and lower animals with animal parasites. A striking illustration of the transplantation of an animal parasite to a new country by lanes of travel is that of *Diphyllbothrium latum*, the broad tape-worm of man, which has been known for several centuries in Europe, in particular in the states bordering the Baltic sea, in Finland, northern Sweden, parts of Russia, Switzerland, and northern Germany. This parasite is also known in Japan. Evidence clearly points to its introduction into North America by immigrants, for in certain parts of Finland and Sweden every inhabitant harbors the worm. The first case in America, reported by Leidy in 1879, was that of a native of Sweden, resident in Pennsylvania but three months.

Although it has been known for many years that the larval stage of the worm lived as a small coiled white worm, in the muscle of certain fishes, in particular, pike, perch, pickerel and trout, it was not until 1917 that Janicki and Rosen demonstrated its complete life history. They showed that the egg passing in the feces from the adult worm hatched into a ciliated form known as a coracidium. This free-swimming animal, when eaten by certain copepoda, in Europe, *Cyclops strenuus*, grows in the body cavity into a small elongated worm after about two weeks. At this time the cyclops, if eaten by the proper fish, transmits the parasite by way of the fish's stomach into its musculature where the larva, known as a plerocercoid, awaits being eaten by a suitable mammalian host. It is obvious that only those who eat raw or undercooked fish could become infested with the parasite, and this explains the distribution of the worm, since it abounds in localities where it is the custom of the people to eat fish raw or

cold smoked. Perhaps the custom dates back to primitive fishermen, who even before the discovery of fire, ate their fresh, raw catches on the banks of lakes and streams.

Finlanders, Scandinavians and Russians, coming to this country, have settled in the middle western lake region and in Manitoba for there they find farming, fishing, trapping and iron mines, which furnish similar occupations to those pursued in their native lands. The ova of *Diphyllbothrium latum* found their way into the lakes at first accidentally; finally as the towns grew in size, they were deposited there through the agencies of modern sewerage systems, in large quantities. Essex, and Essex and Magath have demonstrated three copepods in this region capable of harboring the first larval stage, namely, *Diaptomus oregonensis*, *Diaptomus sicilis* and *Diaptomus siciloides* and Magath proved the presence of the plerocercoids in fishes from these lakes. Since then, these investigators, and Nicholson and Vergeer have shown pike, pickerel and perch from several lakes in northern Minnesota, from lakes in the northern peninsula of Michigan and from lakes in Manitoba and Alberta to be infested. Since most of the lakes involved belong to a chain which drains into Hudson's Bay it is possible some fish obtain their infestation in one lake and migrate to others. These areas correspond to the endemic centers of infestation in man previously established by Nickerson, Riley, Warthin and Magath. Sporadic cases have occurred in other parts of the country through the ingestion of raw fish obtained from these infested waters.

Although dogs take the infestation readily, their part in the spread of the worm may be questioned because ova obtained in their feces have very low vitality as compared with those in the feces of man, and their feces are often subjected to freezing and drying, both of which conditions kill the embryo in the ovum. Feces of dogs enter lakes for the most part accidentally.

The observation has been made that the greatest infestation in fishes occurs in lakes where sewage is emptied from towns in which Europeans who are accustomed to harbor the worm live, and the least infestation in lakes removed from habitation. This supports the contention that the parasite was brought to America

by Europeans and is maintained here largely through their continuing their custom of eating raw fish.

The fact that the larvae are killed in the flesh of fish when subjected to temperatures of -9°C . gives a sufficient cue to prevention of the spread of the parasite in man from a commercial standpoint. Any one may prevent his becoming infested by eating no raw or undercooked fish except it be fish from salt water. Diverting sewage from lakes and streams, unless it is rendered free from viable ova or larvae of *Diphyllbothrium latum*, will do much to prevent the spread and increase of infestation of fish. Fish should not be reared in hatcheries where the water is obtained from polluted streams or lakes.

Although a severe grade of anemia, almost exactly like primary anemia, has been described in persons who harbor *Diphyllbothrium latum*, the condition is very rare in America and occurs only in one person out of 5,000 to 10,000 in Finland, where the anemia is most often encountered. After a most careful evaluation of all factors, Birkeland, in a monograph which is in press, stated the conclusion that the worm acts as a "last straw to break the camel's back" in a person who is constitutionally susceptible to such blood dyscrasias.

The importance of this parasite in North America warrants a thorough educational program regarding it and an immediate attempt to control its development and spread.

T. B. M.

SOCIETY NEWS AND NOTICES

Clinical pathologists will be interested in the following bills which were enacted during the first and second sessions of the Seventy-First Congress:

1. Public Law 361 increases the tariff rate from 45 per centum ad valorem to 55 per centum ad valorem on surgical instruments. This includes many instruments used by clinical pathologists such as hypodermic needles and syringes. Upon some syringes, as Record syringes, the rate has been increased to 70 per centum ad valorem. An increase of 20 per centum ad valorem has been made on many other scientific instruments which are chiefly composed of glass, whether for use in hospitals, laboratories, schools, or colleges. The rate on microscopes and such instruments has been increased 15 per centum ad valorem and on certain electrical devices and x-ray equipment the rate has been increased 5 per centum.

2. Public Law 106 provides for the coördination of public health activities of the Federal Government and Public Law 251 changes the name of the Hygienic Laboratory to the National Institute of Health. Public Law 251 further authorizes the acceptance of gifts by the National Institute of Health for the study, investigation and research in fundamental problems of the diseases of man. Individual scientists other than those connected with the Public Health Service may receive fellowships and may be appointed for duty to the National Institute of Health.

So far as can be learned there are no bills of particular interest to clinical pathologists being carried over to the present Congress.

In reviewing bills which were considered by state legislatures, three from New York State are of particular interest:

1. House Bill 2084, which was defeated, proposed to provide for the examination and licensing of laboratory technicians. A laboratory technician was defined as a person "who renders tech-

nical service and laboratory work designed and intended to be used as an aid in the investigation, prevention, diagnosis and/or treatment of diseases, or ailments of the human body, in a clinical research or public health laboratory or in a laboratory or office maintained by a physician or group of physicians in connection with his or their practice."

2. House Bill 157, which was defeated, was introduced to prohibit scientific experiments on living dogs.

3. House Bill 494, which was passed, provides for (1) the licensing by local health officers of persons, firms or corporations engaging in the business of procuring persons to donate human blood for transfusions, and (2) requiring that donors of blood present a certificate from a registered physician showing a satisfactory physical examination of the donor within ten days of the offering of the blood and his freedom from communicable disease.

The training of lay technicians was discussed by Dr. Walter E. King before the Congress of Medical Education, Medical Licensure, and Hospitals in Chicago in February, 1930. In his talk he summarized information concerning laboratories obtained in a questionnaire sent out by Dr. J. H. Black to medical schools and hospital laboratories. From this source of information it was learned that of eighty-one responding to the questionnaire fifty-four were engaged in training student technicians. There are sixteen colleges and medical schools giving regular courses for laboratory technicians varying from two months to four years, three of which lead to a degree. Dr. King pointed out the need for a better standardization of courses and urged the American Society of Clinical Pathologists to specify the minimum of work for students and the classification of technicians. The importance of didactic teaching as well as of laboratory practice was clearly brought out.

Full information concerning the next annual convention to be held June 8-9, in Philadelphia will be published in the May issue of the JOURNAL. Dr. R. A. Kilduffe, Chairman of the Program Committee, Atlantic City Hospital, is anxious that titles for

papers for the meeting be submitted to him at once, as the programs have become so long that it is necessary to make some selection of titles. No title will be accepted after May 15. Reservations should be made immediately with Hotel Adelphia, headquarters for the A. S. C. P.

At the 1930 convention of the Society it was decided that:

1. The Scientific Meeting and its discussions would not be reported stenographically as heretofore;
2. Discussions from the floor would not be routinely published with the papers;
3. That to be eligible for publication discussion must be given to the Secretary in writing.

These provisions are again called to the attention of the Society in order that those who desire their discussions recorded may properly prepare them for publication.

Those who intend to present papers, and who desire a careful discussion, are urged to furnish the Program Committee with a title and abstract *at the earliest possible moment* as only this can make arrangements for discussion possible. It is obvious that if only the title of the paper is given, or if title and abstract are not in the hands of the Committee until the last moment, any prepared discussion will be difficult to arrange. If any member presenting a paper desires to have a specific individual inaugurate the discussion, and will so advise the Program Committee, such arrangements will be made by the Committee.

Dr. W. S. Thomas, Chairman of the Committee on Exhibits, announces that two awards will be given this year. Reservations for exhibit space should be made early. Exhibits may be entered by non-members of the Society.

Attention is called to the letter sent out by the Research Committee on November 15, and to the postcard sent out by the Secretary on November 24, concerning the technic of the Huppert-Nakayama reaction. It is hoped that a general response to these communications will be made during the year.

Under the arrangements entered into with The Williams & Wilkins Company, part of the income from advertising and excess subscriptions is available for additional pages in the JOURNAL. It is, therefore, to the advantage of the Society to solicit subscriptions from others than members and to let the advertisers in the JOURNAL know that their advertisements are appreciated. Cooperation in this respect is urgently requested. In ordering materials from firms which advertise in the JOURNAL be sure to mention the fact that their advertisements were noted.

Members of the American Society of Clinical Pathologists are urgently requested to pay their annual dues immediately to Dr. A. S. Giordano, Secretary-Treasurer, South Bend, Indiana. This is particularly important at this time as the mailing list for the JOURNAL is made up of those members of the Society who are in good standing in regard to payment of dues.

Dr. I. Davidsohn has resigned the position of Director of Laboratories at Mt. Sinai Hospital in Philadelphia and has accepted the position of Director of Laboratories and Pathologist at the Mt. Sinai Hospital in Chicago.

Dr. Alvin G. Foord has resigned his position as pathologist at the Buffalo General Hospital to accept a position as pathologist for the Pasadena Hospital at Pasadena, California.

Dr. Edward F. Cooke of Houston, Texas died January 8, 1931, after a brief illness.